

Aqueous enzyme assisted oil extraction from oilseeds and emulsion de-emulsifying methods: a review

Article

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22 focusing particularly on recent efforts undertaken to improve process economics by
23 recovering and reusing enzymes.

24

25 **Keywords**

26 aqueous oil extraction, enzyme treatment, oil yield, oil characteristics, emulsion separation

27

28 **1. Introduction**

29 Aqueous enzymatic extraction (AEE) is a promising method for the simultaneous
30 extraction of oil and protein from oilseeds. The products are of superior quality and highly
31 suited to human consumption. In the extraction process, water containing selected enzymes
32 forms the extraction medium used for incubating the oilseeds. When enzymes are not
33 employed, the process is termed as aqueous extraction which invariably results in lower oil
34 yield. The use of enzymes allows separation of targeted extracted components with
35 unchanged properties which can potentially influence, favourably, the final product in
36 terms of taste and smell. Interest in this technological approach has also increased recently
37 due to safety and environmental regulatory concerns. In comparison with solvent
38 extraction, the use of an aqueous medium is much safer, environmental-friendly and
39 economical. In addition, it contributes to a much safer and flexible operation, lower energy
40 consumption and operational costs, and lower capital investment. A variety of temporal
41 crops can be processed, and the extracted oil does not need further refining. Non-toxic meal
42 and value-added fibre and protein are also produced as co-products, due to the milder
43 operating conditions employed. In addition, the aqueous medium allows simultaneous

44 separation of phospholipids from the oil. Therefore, degumming step (in case of oilseeds)
45 is not necessary and the overall cost of processing can be reduced (Latif & Anwar, 2011;
46 Latif *et al.*, 2011; Yang Li *et al.*, 2011; Chabrand & Glatz, 2009; Jung & Mahfuz, 2009;
47 Wu *et al.*, 2009; Soto *et al.*, 2007; Santos & Ferrari, 2005; Gros *et al.*, 2003; Hanmoungjai
48 *et al.*, 2001; Rosenthal *et al.*, 2001; Sineiro *et al.*, 1998; Ksenija *et al.*, 1997; Rosenthal *et*
49 *al.*, 1996)

50 Despite the advantages, the application of AEE is still limited due to long
51 processing time and the high cost spent for the drying process after the enzyme treatment
52 (Shah *et al.*, 2005; Dominguez *et al.*, 1996). The high cost may also be attributed to the
53 enzymes themselves, because a significant amount is required (normally >1% of the weight
54 of the oilseed taken). Further, the non-availability of enzymes on a commercial scale has
55 limited the development of such processes (Rui *et al.*, 2009; Shah *et al.*, 2005). An added
56 problem with AEE is that it is impossible to avoid emulsification of the extracted oil, which
57 requires post extraction de-emulsification to recover and enhance oil yield (Latif & Anwar,
58 2011; Long *et al.*, 2011; Wu *et al.*, 2009; Chabrand *et al.*, 2008; Santos & Ferrari, 2005;
59 Rosenthal *et al.*, 1998; Sineiro *et al.*, 1998a). Addition of suitable enzymes to the cream
60 emulsion may be able to separate the oil, and in this paper, this particular sequence of process is
61 termed as aqueous enzymatic emulsion de-emulsification (AEED).

62 In an earlier review by Rosenthal *et al.* (1996), the principles and mechanisms of:
63 mechanical, solvent, aqueous, and aqueous enzymatic extraction methods have been
64 addressed, besides reviewing the effects of enzymes on plant cell composition and methods
65 employed earlier for de-emulsification. The main purpose of this review is to critically
66 assess the information available to date, in order to conclude whether the enzymatic route

67 is a viable industrial option for any given oilseed. In addition, the other objectives of this
68 review are: to discuss the effect of incubating conditions in AEE on the oil extraction
69 efficiency; to compare AEE with other extraction methods in terms of yields and
70 characteristics of the oils from various oil-bearing materials; to explore methods available
71 to de-emulsify the oil- aqueous phase emulsions that are inevitably formed during
72 extraction; and finally, to explore the possibility of re-using in the enzyme after recovery in
73 order to make the process more cost effective.

74

75 **2. Aqueous enzymatic extraction (AEE) method** Table 1 lists the enzymes used in
76 earlier research. In terms of the dispersion structure, Sineiro *et al.* (1998a) reported that
77 aqueous extraction resulted in oil droplets with spherical shapes in the case of sunflower
78 oil. However, with the use of enzymes, the oil aggregates possessed different shapes with
79 less structured and irregular cell wall surface. Different oils exhibit different properties, and
80 it is reasonable to assume that AEE of different oil-bearing materials result in oil droplets
81 with different characteristics. The enhancement in oil yield with the use of enzymes, i.e.
82 AEE as compared to aqueous extraction without enzymes from various oil-bearing
83 materials are summarized in Table 2. The table also summarizes the differences observed
84 in oil yields between AEE and solvent extraction methods. It is clearly shown that the use
85 of enzymes increases the oil yield, yet it is still lower than the yield when solvent
86 extraction is used. Therefore, numerous studies have been conducted to establish the most
87 suitable enzymes that can be used, either individually or in combination, on various types
88 of oil-bearing materials in order to increase the oil yields.

89

90 2.1. Studies comparing extraction efficiencies using different enzymes

91

92 Figure 1(a) and 1(b) illustrate the flow sheets of AEE for soybean and olive oil,
93 respectively. The types of enzymes added depend on the cellular composition and structure
94 of the oil-bearing material (Passos *et al.*, 2009). According to Rosenthal *et al.* (2001), the
95 use of Alcalase 2.4L (protease) increased the oil yield from heat-treated soybean flour as
96 compared to cellulase, hemicellulase, and pectinase. Similarly, Santos and Ferrari (2005)
97 reported that both Alcalase and Celluclast (cellulase) were able to increase the oil yield
98 from soybeans, with Alcalase giving higher yields. A higher yield in the case of protease
99 (96.0%) as compared to phospholipase (73.4%) was also reported by Jung *et al.* (2009) in
100 the case of extruded soybean flakes. In addition, Lamsal *et al.* (2006) reported that the use
101 of individual cellulase and a mixture of cellulase and protease did not significantly increase
102 the soybean oil yield from extruded soybean flakes (68%); yet the yield increased when
103 individual protease was added (88%). These findings illustrate the specificity of enzymes
104 and enzymatic mixtures for any given oil-bearing material. The presence of protein as a
105 major component in the cell wall of soybean seeds suggests that the oil is released more
106 easily from the cellular matrix by degrading the proteins, which is achieved by the action of
107 protease. In the case of rapeseed, pectin is reported to be the major component of its cell
108 wall (Zhang *et al.* 2007), hence the highest oil yields, up to 85.9% in emulsified form, has
109 been reported when pectinase is used which is significantly greater than the values obtained
110 with other carbohydrases. Zhang *et al.* (2007) also employed a combination of pectinase

111 with cellulase and β -glucanase in a ratio of 4:1:1 to result in the highest yield (91.6%
112 emulsified oil), this marginal enhancement in yield may be attributed to the elimination of
113 other barriers to the release of oil. Similarly, Szydłowska-Czerniak *et al.* (2010) reported
114 that the application of pectolytic enzyme (ROHAPECT PTE) under optimum conditions
115 prior to pressing produced higher rapeseed free oil yield (16.5%) as compared to
116 cellulolytic enzyme (15.5%).

117 Different from oilseeds, addition of enzymes is done on the olive paste in the case of olive
118 fruits, followed by its kneading process as shown in Fig. 1(b). Most studies on extraction of olive
119 oil involved addition of an enzyme mixture consisting mainly pectinase, cellulase, hemicellulase,
120 and other minor enzymes. The studies also reported the inadequacies of these enzymes to extract
121 olive oil if added individually (Aliakbarian *et al.*, 2008; De Faveri *et al.*, 2008; Chiacchierini *et*
122 *al.*, 2007).

123 In general, a better oil extraction yield can be expected when a judiciously chosen
124 mixture of enzymes is used because of possible synergy (Passos *et al.*, 2009). However,
125 according to Rovaris *et al.* (2012), there was no significant difference in soybean oil yields
126 when a mixture of Alcalase 2.4 L and Viscozyme was used as compared to a mixture of
127 Alcalase 2.4 L and Celluclast 1.5 L (29.48% as against 26.82% at pH 4.5; 20.63% as
128 against 20.23% in the case of uncontrolled pH), even though Viscozyme itself is a mixture
129 of enzymes. There was also no significant difference in garlic oil yields upon addition of
130 Viscozyme as compared to addition of individual pectinase, protease, and cellulase as
131 reported by Sowbhagya *et al.* (2009). A similar outcome was reported by Tabtabaei and
132 Diosady (2013) in yellow mustard flour oil extraction when Celluclast 1.5L and Pectinex
133 Ultra SP-L were used, as against Viscozyme L. In addition, the use of Alcalase 2.4L and

134 Protex 7L resulted in highest sesame (Latif & Anwar, 2011) and *Moringa oleifera* (Latif et
135 al., 2011) seed oils, respectively, in comparison with Viscozyme L, Protex 7L, Natuzyme,
136 Kemzyme, and Multifect CX 13L which are essentially mixtures of enzymes (Latif
137 & Anwar, 2011; Latif et al., 2011). Viscozyme, being a mixture of enzymes, was reported
138 to have performed better in the case of sunflower oil extraction, which had been proved by
139 Latif and Anwar (2009). A higher oil yield from bush mango kernel flour was also
140 observed upon addition of Viscozyme (68.0%) as compared to Alcalase (35.0%) and
141 Pectinex (42.2%) (Womani et al., 2008). The different effects of the Viscozyme on oil
142 yields may be due to the nature of different oil-bearing materials and incubating conditions
143 employed.

144 In a different study conducted by Jiang et al. (2010), five different proteases were
145 tested to improve peanut oil yield, and the highest oil yield was obtained when Alcalase
146 was used (73.45%), followed by As1398 (66.36%), Nutrase (60.08%), Protizyme
147 (55.02%), and Protamex (48.89%). A combination of Alcalase with any of these enzymes
148 did not increase the oil yield. Therefore, Jiang et al. (2010) only used Alcalase which
149 reduced the extraction cost, and increased oil yield up to 79.32% under optimum
150 incubating conditions. Similarly, the use of Neutrase 0.8L resulted in marginally lower
151 *Moringa oleifera* oil yield than when its combination with other three enzymes were
152 employed (Abdulkarim et al., 2006). In the case of flaxseed oil extraction conducted by
153 Long et al. (2011), the addition of cellulase, pectinase, and hemicellulase, individually,
154 gave higher yields than β -glucosidase and proteinase. Therefore, these authors used a
155 mixture of cellulase, pectinase, and hemicellulase (1:1:1) which resulted in a higher oil

156 yield of 61.7-66.1% as compared to the oil yield of each individual enzyme. With reference
157 to Table 2, , Zhang *et al.* (2007) reported highest yield of 92.7% in the case of rapeseed oil,
158 however, the oil remained very stably emulsified in the cream. Therefore, an alkaline
159 extraction was conducted by using Alcalase which resulted in protein degradation along
160 with an increase in total oil yield.

161 Based on the above studies, it is not possible to establish conclusively whether it is
162 better to use enzymes individually or in combination, although there are numerous
163 instances where there is a possibility that a mixture can work synergistically. The choice of
164 enzyme depends on the location of the oil within the cellular architecture and the
165 biochemical nature of the components surrounding it. It is therefore necessary, not only to
166 look at the dominant biochemical component holding the cellular matrix together, but also
167 investigate the cellular architecture and examine the specific components which act as a
168 barrier against the release of oil. It is only when both these factors are considered
169 simultaneously, the right enzyme mixture can be identified for a given oil-bearing material.

170

171 2.2. Studies on the use of enzyme as a pre-treatment step prior to extraction

172

173 Recently, the application of enzyme pre-treatment prior to oil extraction has been
174 shown to increase yields (Li *et al.*, 2012). The addition of enzymes as a pre-treatment
175 weakens the cells and facilitate the following oil extraction methods such as mechanical
176 pressing and solvent treatment. Furthermore, the advantage of employing this approach lies
177 in the possibility of avoiding the formation of an oil-in-water emulsion that is very difficult

178 to separate after the extraction processes. The reported enhancement in oil yields with the
179 use of enzyme pre-treatment is summarized in Table 3. In addition to the higher yield,
180 Dominguez *et al.* (1996) also reported that it was easier to extract the sunflower oil
181 remaining in a mass of pre-treated mechanically pressed cake. In the case of Chilean
182 hazelnuts, enzyme pre-treatment resulted in significantly lower residual oil in the meal as
183 reported by Zuniga *et al.* (2003). Overall, these studies indicate that enzyme pre-treatment
184 is applicable to various oil-bearing materials and can be employed prior to both mechanical
185 and solvent extraction methods. The oil yield enhancement is due to the hydrolytic action
186 of the enzymes on the cell wall and membrane components which facilitate subsequent oil
187 release.

188

189 2.3. Studies on pre-treatment step prior to enzymatic extraction

190

191 Some studies have highlighted potential pre-treatment methods, which are not
192 necessarily enzyme-based that could be followed up by AEE as summarized in Table 4. In
193 the case of high pressure processing as reported by Jung and Mahfuz (2009), the use of
194 high pressure induced protein aggregation yet it was further hydrolyzed by protease, thus
195 facilitated oil removal. On the other hand, Shan Liu *et al.* (2011) reported that ultrasound
196 generated cavitations which accelerated the leaching out of cellular components including
197 oil. The use of extrusion prior to AEE has been extensively studied by Jung and Mahfuz
198 (2009), Jung *et al.* (2009), and Wu *et al.* (2009). According to these authors, protein
199 aggregates are formed during extrusion but these entrap or interact with the oil. The

200 interactions could then be disrupted by the use of protease, which result in increasing the
201 oil and protein yields. These studies have shown the potential of AEE assisted by other pre-
202 treatment methods to increase oil yields.

203

204 2.4. Factors affecting the efficiency of enzymatic extraction

205

206 Table 5 summarizes the maximum oil yields resulting from various oil-bearing
207 materials as influenced by the selected and optimized incubating conditions. The key
208 factors affecting the efficiency of AEE will be discussed separately, below.

209

210 2.4.1. Particle size of the oil-bearing materials

211 Most of the early studies did not consider the particle size of the oil-bearing
212 material as a key factor influencing extraction efficiency (Passos *et al.*, 2009; Rosenthal *et*
213 *al.*, 2001). Theoretically, the lower the particle size, the higher the oil yield for a given set
214 of extraction conditions, which is attributable to higher cell wall disruption during size
215 reduction as well as the lower diffusion path length for both enzymes and cellular
216 components. However, according to Passos *et al.* (2009), materials with high oil content
217 but exhibiting a weak structure, may collapse and lose their microporosity when treated
218 with solvents, which can result in non-uniform percolation and be detrimental to extraction
219 efficiency. In addition, grinding of materials with high oil content into very low particle
220 sizes may cause the particles to adhere, as reported by Nyam *et al.* (2009a) in the case of
221 Kalahari melon seeds. Therefore, in industry, starting materials with very low particle size

222 are not recommended and there appears to be an optimum size. This illustrates the
223 importance of selecting the right particle size prior to extraction as had been done by some
224 authors. Sineiro *et al.* (1998a) used ground soybean and sunflower seeds having mean
225 particle size <0.2 mm. The grape seeds used by Passos *et al.* (2009) were grouped into
226 different particle size ranges (in mm): <0.50, 0.50-0.60, 0.60-0.71, 0.71-1.0, 1.0-1.4, 1.4-
227 2.0, and >2.0, and increment in oil yield was observed at lower particle sizes. In the case of
228 linseed oil, Gros *et al.* (2003) reported no oil recovery from whole linseed kernels, because
229 the substrate was not accessible to the enzymes added. Instead, the hull broke down and the
230 kernels expanded due to hydration. On the other hand, when the kernels were crushed to
231 form different particle sizes including fine powders, the yields improved, particularly after
232 applying hydraulic pressures (Gros *et al.*, 2003). Similarly, in the case of soybean, the use
233 of flour resulted in 24% higher yield than the flakes (Jung *et al.*, 2009), while 31% yield
234 enhancement was reported by Rosenthal *et al.* (1998) when the particle size was reduced
235 from 400 μm to 100 μm .

236

237 2.4.2. Enzyme/substrate ratio

238 Higher enzyme concentration leads to greater interaction between the enzyme and
239 substrate, thus promoting cell wall degradation and rupturing more peptide bonds (Teixeira
240 *et al.*, 2013; Jiang *et al.*, 2010; Dominguez *et al.*, 1996). However, too high enzyme
241 concentration may result in bitterness and off flavours, as reported by Jiang *et al.* (2010),
242 possibly due to the extraction of undesirable components. Most authors have reported
243 similar trends where the oil yield increased up to certain enzyme concentration only,

244 followed by steady or decreased rate which may be due to saturation of the substrates
245 (Jiang *et al.*, 2010), or caramelization of soluble sugars that limit oil release (Zuniga *et al.*,
246 2003). In general, the actual concentration used will depend on process economics
247 especially the cost of enzymes (Long *et al.*, 2011; Zhang *et al.*, 2007), and the quality of
248 the oil extracted.

249

250 *2.4.3. Ratio of water to oil-bearing material*

251 The water used in AEE not only serves as an extraction medium but also enters the
252 oil-bearing material and modifies its water activity. The resulting moisture content of the
253 oil-bearing material can assist hydrolytic reaction, diffusion, and mobility of the enzymes
254 and products (Yang Li *et al.*, 2011; Zhang *et al.* 2007; Sineiro *et al.*, 1998a; Dominguez *et*
255 *al.*, 1996). On the other hand, very low moisture content results in the formation of thick
256 suspensions which can prevent the enzymes from effectively penetrating into the substrate
257 (Zhang *et al.*, 2007). Sineiro *et al.* (1998a) reported that only certain 'areas' in sunflower
258 kernels were degraded by enzymes at low moisture content. Although, materials with
259 higher water activity demonstrate higher extraction efficiency (Soto *et al.*, 2007), the
260 presence of excessive moisture content in the oil-bearing material can decrease the
261 concentration of enzymes and substrates, and have an adverse effect on extraction (Yang Li
262 *et al.*, 2011; Zhang *et al.*, 2007; Dominguez *et al.*, 1996). Therefore, selection of
263 appropriate moisture content is critical for the success of AEE.

264

265 *2.4.4. pH of extraction medium*

266 The pH at which enzymes attain maximum activity varies with the enzyme. In most
267 earlier studies, the pH value of the solution, be it for soaking pre-treatment or extraction
268 itself, was set at a value corresponding to maximum enzyme activity (Latif & Anwar, 2011;
269 Jung & Mahfuz, 2009; Wu *et al.*, 2009; Abdulkarim *et al.*, 2005; Rosenthal *et al.*, 2001;
270 Sineiro *et al.*, 1998). However, the optimum pH of a number of enzymes is in the range of
271 the isoelectric pH of proteins which depends on the nature of the oilseeds; since proteins
272 are highly insoluble in this range of pH, oil release may get inhibited. Therefore, the pH
273 value employed must not only be conducive for the action of enzymes but it should also be
274 remote from protein isoelectric point (Tabtabaei & Diosady, 2013; Wu *et al.*, 2009; Sineiro
275 *et al.*, 1998; Rosenthal *et al.*, 1996). This is yet another reason why many authors
276 considered using a mixture of enzymes which demonstrates high activity at pH values
277 remote from the isoelectric point and remain effective for oil extraction. The enzymes are
278 able to solubilize and hydrolyze the proteins besides disrupting other polysaccharide
279 constituents which facilitate oil release (Rovaris *et al.*, 2012; Latif & Anwar, 2011; Passos
280 *et al.*, 2009). Long *et al.* (2011) had used a mixture of cellulase, pectinase, and
281 hemicellulase (1:1:1) at pH 4.5-5.0 which resulted in highest flaxseed oil yield (73.9%) as
282 compared to oil yield of each individual enzyme. In the case of soybean oil, at pH 4.5,
283 Rovaris *et al.* (2012) used a mixture of Alcalase 2.4L and Celluclast 1.5L which resulted in
284 26.82% oil (20.63% in the case of uncontrolled pH), and a mixture of Alcalase 2.4 L and
285 Viscozyme which resulted in 29.48% oil (20.23% in the case of uncontrolled pH). A
286 number of studies have also used ProtizymeTM for the AEE (Jiang *et al.*, 2010; Gaur *et al.*,
287 2010; Sharma *et al.*, 2002). ProtizymeTM, being a mixture of proteases, possess different

288 optimum pH which allowed selection of any incubating pH sensitive to the isoelectric point
289 of the major protein fraction of the seeds. Overall, proper pH selection critically influences
290 yields of oil and other components in AEE .

291

292 2.4.5. Incubation temperature

293 Besides being active over a narrow range of pH, enzymes also active over a narrow
294 temperature interval. According to Rui *et al.* (2009), the optimum temperature range for
295 enzymatic hydrolysis is between 40-55 °C, thus many authors employ AEE temperatures
296 which fall within this range. In practice, one often prefers to use the lowest possible
297 temperature yielding adequate activity (Passos *et al.*, 2009). In the case of olive fruits, a
298 lower temperature of 30 °C was found to be favourable especially to preserve the oil
299 quality (Aliakbarian *et al.*, 2008; De Faveri *et al.*, 2008; Ranalli *et al.*, 2003; Garcia *et al.*,
300 2001; Ranalli *et al.*, 1999). Gros *et al.* (2003) also used a temperature of 34 °C for similar
301 reason in linseed oil extraction. A significant effect of temperature on oil yield was
302 reported by Sharma *et al.* (2002), where highest peanut oil yield was observed at 40 °C, but
303 it decreased significantly when the temperature was reduced to 37 °C. According to Zúniga
304 *et al.* (2003), at temperatures greater than 45 °C, enzymatic hydrolysis begins to decrease
305 due to enzyme inactivation which leads to lower oil yield. The oil release from the cells
306 may also be limited due to presence of soluble sugars in the composition which can
307 undergo caramelization during the drying stage. Therefore, similar trends were reported
308 from most of the conducted studies, where the oil yield increased up to certain temperature
309 only, followed by steady or decreased rate afterwards. Thus, besides the oil yield, the oil

310 quality characteristics must also be taken into consideration when selecting AEE

311 temperature.

312

313 2.4.6. Incubation time

314 According to Jiang *et al.* (2010), Abdulkarim *et al.* (2006), Santos and Ferrari
315 (2005), and Dominguez *et al.* (1996), degradation of cell wall components can be enhanced
316 by prolonging the incubation time. Passos *et al.* (2009) also reported that the use of an
317 enzyme mixture of cellulase, protease, xylanase, and pectinase for 120 hr resulted in 3.8%
318 higher yield as compared to 24 hr of incubation time. However, this time duration (i.e. 120
319 hr) is far too long to be acceptable in practice (Passos *et al.*, 2009), lower oil quality may
320 result (Jiang *et al.*, 2010), leading to high energy usage and production of undesirable
321 products (Abdulkarim *et al.*, 2006). In addition, Rui *et al.* (2009) highlighted that longer
322 incubation time of AEE in relation to other solvent extraction methods is one of the
323 disadvantages of AEE. In some cases, the oil yield decreased after a certain incubation
324 period because the whole substrates have reacted with the enzymes; leaving negligible
325 substrates left for further enzymatic reaction to take place (Zhang *et al.*, 2007). On the
326 whole, these studies have shown that although oil yield may increase with time, the rate of
327 increase may be far too slow to warrant extended operations, and the oil quality may also
328 get compromised.

329

330 2.4.7. Agitation rate

331 According to Rosenthal *et al.* (1998) and Sineiro *et al.* (1998a), agitation assists in
332 mixing and additional rupture of the cell wall, and agitation rate is one of the factors
333 affecting the disruption of cell wall. Abdulkarim *et al.* (2006) reported that the agitation
334 rates of 50 and 80 rpm were not adequate to separate the *Moringa oleifera* oil from other
335 seed components, thus resulted in lower oil yield than at 120 rpm. At this agitation rate of
336 120 rpm, bigger oil droplets were observed to accumulate at the surface which enabled
337 easier separation. A similar observation was reported at 80 rpm in extraction of peanut oil
338 (Sharma *et al.*, 2002) and at 100 rpm in the extraction of Kalahari melon seed oil (Nyam *et*
339 *al.*, 2009a). On the other hand, the use of higher speeds leads to higher energy consumption
340 and cost (Rosenthal *et al.*, 1998), besides resulting in the formation of a more stable oil-
341 aqueous phase emulsion that is difficult to separate (Nyam *et al.*, 2009a; Abdulkarim *et al.*,
342 2006; Sharma *et al.*, 2002, Hanmoungjai *et al.*, 2000). These studies highlight the
343 importance of selecting appropriate agitation rate that will result in the highest oil yield
344 possible, considering both the oil recovered and emulsion stability at the end of the AEE
345 process.

346

347 2.5. Multi factorial studies on AEE

348

349 A number of authors have employed statistical methods to indicate the relative
350 importance of the AEE parameters listed above. According to Rosenthal *et al.* (2001),
351 soybean oil yield was significantly influenced by the type of enzyme used, the particle size
352 of the ground seeds, the ratio of water to oil-bearing material, and the interaction between

353 the two latter parameters. However, according to Hanmoungjai *et al.* (2001), only the
354 enzyme concentration had the most significant effect on the extraction of rice bran oil,
355 while both the incubation time and temperature did not significantly affect the oil yield.
356 Different AEE parameters used for other samples such as bayberry kernels (Zhang *et al.*,
357 2012), kalahari melon seeds (Nyam *et al.*, 2009a), palm fruit (Teixeira *et al.*, 2013), peanuts
358 (Jiang *et al.*, 2010), and pine kernels (Yang Li *et al.*, 2011) also had different degree of
359 significant effect on oil yield. These studies show that it is almost impossible to generalize
360 which factor is important and which is not, for a given material. It is necessary to undertake
361 an experimental investigation before designing and scaling up an AEE process.

362

363 **3. De-emulsification methods for aqueous enzymatic process (AEED)**

364 When oil is extracted into an aqueous enzymatic phase, it inevitably forms an emulsion,
365 which is often difficult to separate because of the added stability imparted by the
366 interfacially active cellular components which are also extracted in the same process. It is
367 therefore necessary to carefully consider the techniques employed to separate the oil,
368 because the final yield and oil quality, and the economic viability of the process, will
369 depend critically on de-emulsification steps. When AEE is followed by a centrifugation
370 step, besides oil, other fractions recovered include a skim and a cream emulsion (Figure
371 1(a)). The cream emulsion is very stable due to its protein content which acts as an
372 excellent emulsifier. Addition of suitable enzymes to the cream emulsion may be able to
373 separate the oil, and in this paper as had been mentioned earlier, this particular sequence of
374 process is termed as aqueous enzymatic emulsion de-emulsification (AEED). The enzymes

375 used in the AEED processes were also listed in Table 1. In this method, the enzymes added
376 to the cream emulsion hydrolyze the interfacial proteins, thus reducing their molecular size
377 and decreasing the rigidity of the oil droplet interface. The enzymes also remove the high
378 molecular weight polypeptides which may occupy the emulsion interface and further
379 reduce the interfacial membrane thickness. These enzymatic reactions lead to greater oil
380 droplet coalescence and assist in free oil release (Tabtabaei & Diosady, 2013; Raghavendra
381 & Raghavarao, 2010; Chabrand & Glatz, 2009; Jung & Mahfuz, 2009; Marina *et al.*, 2009;
382 Wu *et al.*, 2009; Chabrand *et al.*, 2008). The original enzymes used in the AEE may also be
383 carried out into the cream emulsion and assist hydrolytic reactions if suitable incubating
384 conditions were employed (Chabrand & Glatz, 2009; Jung *et al.*, 2009). The free oil yield
385 is commonly expressed as a percentage based on the initial weight of the cream emulsion.

386 In the case of oil-bearing coconut milk, the emulsion needs to be destabilized in
387 order to obtain virgin coconut oil as shown in Figure 1(c). According to Jena and Das
388 (2006), Garcia *et al.* (2005), Tangsuphoom and Coupland (2005), and Balasundaresan *et al.*
389 (2002), coconut milk emulsion is low in stability due to its high fat content and the
390 presence of coconut proteins (~65% is globulin known as cocosin) with low emulsifying
391 properties. Therefore, these authors noted that the separation was not too challenging and
392 concluded that the oil droplets were prone to undergo aggregation and tended to separate.
393 In contrast, Marina *et al.* (2009), Tangsuphoom and Coupland (2008), Peamprasart and
394 Chiewchan (2006), and McGlone *et al.* (1986) reported that a coconut cream emulsion was
395 highly stable due to presence of natural phospholipids and coconut proteins (mainly
396 globulins and albumins) which requires extra energy to be destabilized. It is not uncommon

397 to find such conflicting reports in literature, in this area, which is principally because, most
398 papers do not take a holistic view on the whole process. Whether the downstream de-
399 emulsification is challenging or not depends on the process conditions employed during
400 AEE. If the conditions employed are such that the emulsion formed is very stable, then the
401 de-emulsification will naturally become challenging. On the other hand, careful process
402 design upstream, and use of conditions that do not favour the formation of a stable
403 emulsion whilst releasing significant yields of oil, will simplify de-emulsification and
404 enhance free oil yields and oil quality.

405

406 3.1. Studies comparing different enzymes for de-emulsification of cream emulsion

407

408 Table 5 summarizes the types of enzymes and the incubating conditions used in
409 AEED methods for maximum free oil yields. In the case of yellow mustard flour, Tabtabaei
410 and Diosady (2013) reported that Protex 6L possessed greater efficiency in the de-
411 emulsification process, as compared to other proteases and carbohydrases tested. Lipomode
412 (Phospholipase A2), being one of the carbohydrases, resulted in the production of
413 lysophospholipids which is an emulsifier, thus increased the emulsion stability and
414 decreased the free oil yield. Lysophospholipids also present in small amount in G-ZYME
415 G999, resulted in an insignificant increase in the free oil yield. In the case of soybean oil,
416 Lamsal and Johnson (2007) concluded that the use of Phospholipase C resulted in higher
417 free oil yield ($73\pm 5\%$) as compared to the mixture of LysoMaxTM and G-ZYME G-999 at
418 1:1 ratio ($68\pm 9\%$) under the optimum pH and temperature of the enzymes. Wu *et al.* (2009)

419 have also reported that the use of enzymes shown in Table 5 at their optimum pH and
420 temperature resulted in total de-emulsification of the cream emulsions, either the enzymes
421 had been used individually or in combination, or sequentially. These studies indicated that
422 the free oil yield depends on the stability of the cream emulsion which is mainly affected
423 by the AEE, besides the incubating conditions of the AEED which are discussed below.

424

425 3.2. Factors affecting the efficiency of enzymatic de-emulsification

426

427 3.2.1. Enzyme concentration

428 Generally, the use of higher enzyme concentration resulted in higher free oil yield.
429 According to Jung *et al.* (2009), at 25 °C, the use of Protex 6L resulted in higher free
430 soybean oil yield of 96% at 2.5% (w/w) concentration when compared to a 85-89% yield
431 while employing enzyme at 1.25% (w/w). Similarly, Wu *et al.* (2009) reported that free
432 soybean oil yield increased with increasing enzyme concentration starting from 0.2%
433 (w/w). In this study, when the LysoMax™ enzyme was used at a concentration lower than
434 0.2% (w/w), the enzyme modified soybean phospholipids and caused the production of an
435 emulsifier known as lysolecithin. This emulsifier enhanced the stability of the cream
436 emulsion and therefore resulted in lower free oil yield. In addition, according to Wu *et al.*
437 (2009), increasing the LysoMax™ enzyme concentration did not increase the oil droplets
438 size. These authors also reported that in the concentration range of 0.2-2.0% (w/w), the use
439 of Protex 51FP resulted in higher free oil yield as compared to the LysoMax™ which
440 indicated the dominant role of soybean protein in stabilizing the cream emulsion.

441

442 3.2.2. pH value

443 As had been discussed earlier (section 2.4.4), different enzymes possess different
444 optimum pH where maximum activity is observed. Therefore, most studies employed the
445 optimum pH of the enzyme used in order to obtain the highest free oil yield (Table 5). In
446 the case of soybean oil, according to Wu *et al.* (2009), the oil droplet size and free oil yield
447 increased when the pH was lowered to 4.5, but not lower than 4.0. At the pH of 4.5, which
448 is the isoelectric point of soy protein, electrostatic repulsion between oil droplets decrease,
449 thus further enhancing oil droplets coalescence, formation of larger oil droplets, and higher
450 free oil yield (Wu *et al.*, 2009). In a study conducted by Chabrand and Glatz (2009), the
451 authors reported as high as 83% free soybean oil yield when the pH of the cream emulsion
452 was reduced to pH 4.5, and addition of enzyme (G-ZYME G999) at this similar pH
453 increased the free oil yield up to 100%. Similarly, Wu *et al.* (2009) reported that the use of
454 G-ZYME G999 and Protex 50FP separately at pH 4.5 resulted in 100% free oil yield.
455 These authors suggested that the combination of enzymatic reaction and pH reduction leads
456 to coalescence of the oil droplets and formation of much bigger droplets than when
457 enzymes are not used. Chabrand and Glatz (2009) had also reported the use of high pH on
458 the free soybean oil yield. At pH 9, only 2% of free oil yield was recovered. With the use
459 of enzymes (i.e. AEED) at pH 8 which was the original pH of the cream emulsion, no free
460 oil yield was obtained. Similarly, Wu *et al.* (2009) reported that the free soybean oil yield
461 decreased when the pH was increased beyond pH 4.5 up to pH 8. Therefore, the
462 significance of enzymes addition at suitable pH values for higher free oil yield is clear.

463

464 *3.2.3. Incubation time and temperature*

465 Similar to the pH value, different enzymes possess different optimum temperature
466 where maximum activity is observed. Therefore, most earlier studies employed the
467 optimum temperature reported for the enzyme used in order to obtain highest free oil yield
468 (Table 5). Jung *et al.* (2009) reported the effect of different de-emulsification temperatures
469 and times on the free soybean oil yield when Protex 6L was used. Prolonged incubation
470 time from 2 min to 90 min enhanced the free oil yield from 86% to 100% at 65 °C.
471 However, the incubation time did not affect the free oil yield at lower temperatures of 25
472 °C and 50 °C. Increment of temperature from 50 °C to 65 °C also increased the free oil
473 yield from 90% to 100% after incubation for 90 min. In the case of coconut milk de-
474 emulsification, Raghavendra and Raghavarao (2010) reported a higher free oil yield when
475 the use of enzyme was followed by chilling and thawing. In this case, a higher free oil yield
476 of 94.5% was reported at a higher temperature of 37 °C as compared to 91.0% yield at 25
477 °C, because according to these authors, most enzymes possess an optimum temperature of
478 37 °C. In addition, chilling resulted in packed oil bodies which are easier to separate
479 (Raghavendra & Raghavarao, 2010).

480 It is also possible to demulsify without the use of enzymes as reported by Jung *et al.*
481 (2009). In this study, the increase in temperature from 50 °C to 65 °C increased the free oil
482 yield from 75% to 94%. According to the authors, the significant increase in free oil yield
483 may be due to the action of remaining protease in the cream emulsion which was carried
484 out from the AEE. In the case of yellow mustard flour, Tabtabaei and Diosady (2013)

485 subjected the emulsion recovered after AEED process to an alkaline treatment which
486 resulted in higher oil yield than AEED alone.

487 Other processing parameters such as shaking, de-canting, and stirring may also
488 influence de-emulsification efficiency (Jung *et al.*, 2009).

489

490 **4. Oil characteristics**

491 Most authors have reported the effects of extraction methods on the oil characteristics
492 which are summarized in Table 6. With reference to the table, the oil yields from most of
493 the enzyme treatments were lower in oxidative deterioration and rancidity, indicated by the
494 lower free fatty acids and peroxide values as compared to the yields from solvent
495 treatments. It was assumed that the high temperature used during the solvent extraction
496 resulted in lower oxidative quality of the oils (Latif *et al.*, 2011; Latif & Anwar, 2011; Latif
497 & Anwar, 2009; Latif *et al.*, 2008). The peroxide value of rice bran oil extracted by solvent
498 was also higher than that extracted enzymatically, but the difference was too small to the
499 limit industrial application (Hanmoungjai *et al.*, 2001). In contrast, Kalahari melon seed oil
500 from AEE process gave higher free fatty acid and peroxide value than solvent extracted oil.
501 This may be due to the lipase activity in the seeds during the initial heating in the case of
502 AEE process (Nyam *et al.*, 2009).

503 With reference to Table 6, some of the enzymatically extracted oils gave higher
504 iodine value (IV) than aqueous and solvent extracted oils. Hanmoungjai *et al.* (2001) and
505 Long *et al.* (2011) reported that the higher IV indicated higher polyunsaturated fatty acid
506 content which therefore suggested a higher antioxidant activity. In addition, highest total

507 tocopherols was observed in most seed oils obtained from the AEE, followed by aqueous
508 and solvent extracted oils. It was suggested that the higher temperature employed in the
509 solvent treatment reduced the tocopherol content in the oil (Latif *et al.*, 2011; Latif &
510 Anwar, 2011). The total tocopherols in olive oils reported by Ranalli *et al.* (2001) and
511 Ranalli *et al.* (2003) were also higher when AEE was employed as compared to aqueous
512 extractions without enzymes. In contrast, Nyam *et al.* (2009) reported lower total
513 tocopherol content in the Kalahari melon oil obtained by AEE than solvent extraction
514 method. This may be due to the production of components during the digestion process in
515 the AEE that can influence the amount of non-saponifiable matter, including tocopherols
516 (Gunstone, 2000),

517 In terms of total phenolic content, the values varied with different oil-bearing
518 materials, extraction methods employed, and the types of enzymes used in the AEE
519 process. In the case of olive oil, AEE resulted in higher total phenolic content than the
520 aqueous extractions without enzymes. This may be due to cell wall hydrolysis by the
521 enzymes used which further assists partitioning of the phenolics into the oil. The phenolic
522 content positively influences oxidative stability, shelf life, nutritional, sensory, and health
523 properties of the olive oil, besides flavour which got a greater sensory score (Latif &
524 Anwar, 2009, 2011; Aliakbarian *et al.*, 2008; Ranalli *et al.*, 2003; Ranalli *et al.*, 1999;
525 Ranalli & De Mattia, 1997). Najafian *et al.* (2009) also reported that at higher enzyme
526 concentration, the phenolic content increased whilst the oil turbidity decreased, which may
527 be due to the enzymatic effect in reducing the amount of colloidal particles.

528 In terms of the fatty acid compositions (FAC), most authors reported similarities
529 between the oils obtained from solvent and enzymatic extraction methods (Teixeira *et al.*,
530 2013; Li *et al.*, 2012; Zhang *et al.*, 2012; Latif *et al.*, 2011; Latif & Anwar, 2009, 2011;
531 Jung *et al.*, 2009; Nyam *et al.*, 2009, 2009a; Latif *et al.*, 2008). In a study conducted by Rui
532 *et al.* (2009), the FAC of the pitaya oil obtained from microwave-pre-treated enzyme
533 treatment was similar to the recommended FAC by the US dietary standard. Rui *et al.*
534 (2009) suggested that microwave irradiation enhanced volumetric swelling of the cells in
535 the seed kernels which caused cell walls rupture, while the enzymes hydrolyzed the cell
536 wall and the bonds between the protein or pectin. A combination of these methods led to
537 extraction of pitaya oil with varying fatty acid types as compared to other methods. In the
538 case of flaxseed oil, Long *et al.* (2011) reported that the oil yield from enzyme-pre-treated
539 ultrasonication possessed higher monounsaturated and polyunsaturated fatty acids than the
540 flaxseed oil obtained by solvent extraction. According to the authors, the use of water
541 allowed diffusion of water-soluble components instead of the oil. Therefore, the oil
542 possessed approximately similar FAC as the original flaxseed oil (Long *et al.*, 2011).

543 In addition to the characteristics listed in Table 6, the colour intensity of oil had also
544 been reported in some studies based on red and yellow units; higher values of these units
545 correspond to higher colour intensity. In the case of *Moringa oleifera* seeds, according to
546 Latif *et al.* (2011) and Abdulkarim *et al.* (2006), the different enzymes used in the AEE
547 processes act on different components of the seeds which resulted in oil yields having
548 different colour intensity. However, the difference was more significant between the oil
549 obtained by AEE and solvent extraction methods, which is similar to the results reported by

550 Nyam *et al.* (2009) and Latif *et al.* (2008) for Kalahari melon and canola seed oil,
551 respectively. The solvent-extracted oil had higher colour intensity which may due to the
552 pigments extracted by the solvent into the oil, such as carotenes and chlorophylls. The oil
553 obtained from AEE process may not need refining due to low colour intensity which
554 reduces the processing costs (Latif & Anwar, 2009; Nyam *et al.*, 2009; Latif *et al.*, 2008;
555 Abdulkarim *et al.*, 2006, Abdulkarim *et al.*, 2005).

556 Besides the colour of the oils, the sterols were also significantly lower in oil
557 obtained by AEE than solvent extracted oil, which suggests the ability of the solvent used
558 to extract lipid-soluble components (Nyam *et al.*, 2009). In addition to these characteristics,
559 Sowbhagya *et al.* (2009) reported that the use of enzymes as a pre-treatment prior to steam
560 distillation or hydrodistillation resulted in garlic oil with higher concentration of dithiins
561 which possess health benefits and highly desirable from a nutraceutical point of view. In
562 the case of soybean oil, with the use of enzymes, Jung *et al.* (2009) reported lower
563 phosphorus content (<200ppm) which comply with the specification of the National
564 Oilseed Processors Association trading rules for crude degummed soybean oil. In a study
565 done by Ranalli *et al.* (1999), the Cytolase 0 enzyme used in olive oil extraction was
566 harmless and water-soluble. Therefore, after the enzyme exerted all its effects on oil
567 extraction, it came out into the water (i.e. olive juice) and left no residue in the oil. Thus the
568 olive oil composition was not modified.

569 In extraction of virgin coconut oil from coconut milk emulsion, a combination of
570 AEED, chilling, and thawing for the coconut milk destabilization resulted in highest
571 creaming index as compared to other destabilization methods which indicated faster oil

572 droplets movement and higher droplets aggregation. As compared to commercial coconut
573 oil sample, the coconut oil possessed higher caprylic (9.4%), capric (6.3%), and medium
574 chain (69.7%) fatty acids. These fatty acid types are known to impart health benefits, and
575 contribute to higher oxidative stability to the oil itself. In addition, the resulting coconut oil
576 was also lower in acid value (0.27%) which also corresponds to lower free fatty acids, as
577 compared to the commercial coconut oil (0.91%). The free fatty acids are responsible for
578 undesirable flavour in the oil. Therefore overall, the coconut oil obtained from AEED
579 followed by chilling and thawing seems to possess greater oxidative stability, and the
580 attributes measured were within the Asian and Pacific Coconut Community standards
581 (Raghavendra & Raghavarao, 2010).

582 Overall, enzyme based extraction methods result in oils with better characteristics
583 as compared to oil obtained from solvent and aqueous extraction methods. Therefore,
584 further studies are desirable to enable industrial application by scaling up.

585

586 **5. Potentials for re-using enzymes in enzymatic extraction methods**

587 Rosenthal *et al.* (1996) highlighted the possible alternatives for improvement of aqueous
588 extraction, including the use of enzymes (i.e. AEE), the optimization of both extraction and
589 de-emulsification processes, utilization of membrane technology, and the potential of water
590 recycling (i.e. enzyme recycling in the case of AEE). Enzyme recycling may assist in
591 reducing the cost of AEE which bears the potential to compete with conventional
592 extraction method based on the market price commanded by the oil (Nyam *et al.*, 2009a)

593 According to Jung *et al.* (2009), after conducting AEE (Protex 6L) to produce
594 soybean oil, the aqueous phase recovered contained 84.7% of the remaining Protex 6L
595 activity. After separation, a major part of this enzyme activity was recovered in the skim
596 fraction (Jung *et al.*, 2009). Similarly, 100% of Protex 6L activity remained in the skim
597 fraction in a study conducted by Chabrand and Glatz (2009). These findings indicate the
598 possibility of recovering and re-using the skim fraction as a source of water and enzyme at
599 the upstream end of the process (Jung *et al.*, 2009). In addition, Jung *et al.* (2009) reported
600 lower Protex 6L activity in the cream emulsion, yet adequate to increase the free oil yield
601 with the use of suitable incubation time and temperature. Droplet coalescence was also
602 promoted by the gentle stirring during the incubation of the cream emulsion (Jung *et al.*,
603 2009).

604 Studies concerning the enzyme recycling were conducted in order to improve
605 process economics and lower the environmental impact of the process. Another method
606 which has gained recent interests is the enzyme immobilization, where the enzymes are
607 separated from the treated products before being re-used. It was reported that the separated
608 enzymes possessed enhanced stability (Long *et al.*, 2011; Wan *et al.*, 2008; Roy *et al.*,
609 2004). The increasing demands on enzyme-based methods have resulted in production of
610 more enzymes at lower production costs (Roy *et al.*, 2004; Mondal *et al.*, 2003; Sharma *et*
611 *al.*, 2003; Chase, 1994).

612

613 **6. Concluding remarks**

614 This review has highlighted the main process, advantages, and disadvantages of AEE and
615 AEED as alternative methods for conventional solvent based extraction methods. In order
616 to enhance the oil yield, a combination of AEE with other non-enzymatic processing
617 methods prior to, or after AEE, has been widely conducted and relevant studies have been
618 reviewed in this paper. The process factors influencing AEE and AEED efficiencies, as
619 well as the oil characteristics, have also been discussed. On the whole, the process factors
620 are correlated with each other, and statistical optimization is currently the best solution for
621 investigating the interacting effects between the contributing factors for obtaining highest
622 oil yield with favourable quality. The high cost of enzymes and production of lower oil
623 yield than that of solvent extraction method have been the major drawbacks of AEE
624 process. Despite the problems, the interest in this method for oil and protein extraction has
625 progressively increased due to the perceived environmental advantages.

626

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632

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919 **Figure caption**

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921 **Fig. 1.** Flow sheet for (a) production of extruded soybean oil by aqueous enzymatic
922 extraction and free soybean oil recovery by aqueous enzymatic emulsion de-emulsification
923 method (Adapted from Lamsal and Johnson, 2007; Jung *et al.*, 2009; Wu *et al.*, 2009;
924 Chabrand and Glatz, 2009); (b) production of olive oil by aqueous enzymatic extraction
925 with different post-treatments (Adapted from Ranalli *et al.*, 1999; Garcia *et al.*, 2001;
926 Ranalli *et al.*, 2001; Ranalli *et al.*, 2003; De Faveri *et al.*, 2008; Najafian *et al.*, 2009); and
927 (c) production of virgin coconut oil by aqueous enzymatic emulsion de-emulsification
928 method (Adapted from Raghavendra and Raghavarao, 2010).

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930 **Table captions**

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932 **Table 1. Commercial enzymes used for aqueous enzymatic extraction (AEE) and**
933 **aqueous enzymatic emulsion de-emulsification (AEED) processes: descriptions and**
934 **compositions.**

935 **Table 2. The oil yield enhancement with the use of enzymes, and the oil yield**
936 **difference between the enzyme and solvent extraction methods.**

937 **Table 3. Enhancement in oil yield due to presence of enzyme pre-treatment prior to**
938 **the extraction method, as compared to the extraction method alone.**

939 **Table 4. The advantages of the use of pre-treatments (non-enzymatic) prior to the**
940 **enzymatic extraction method.**

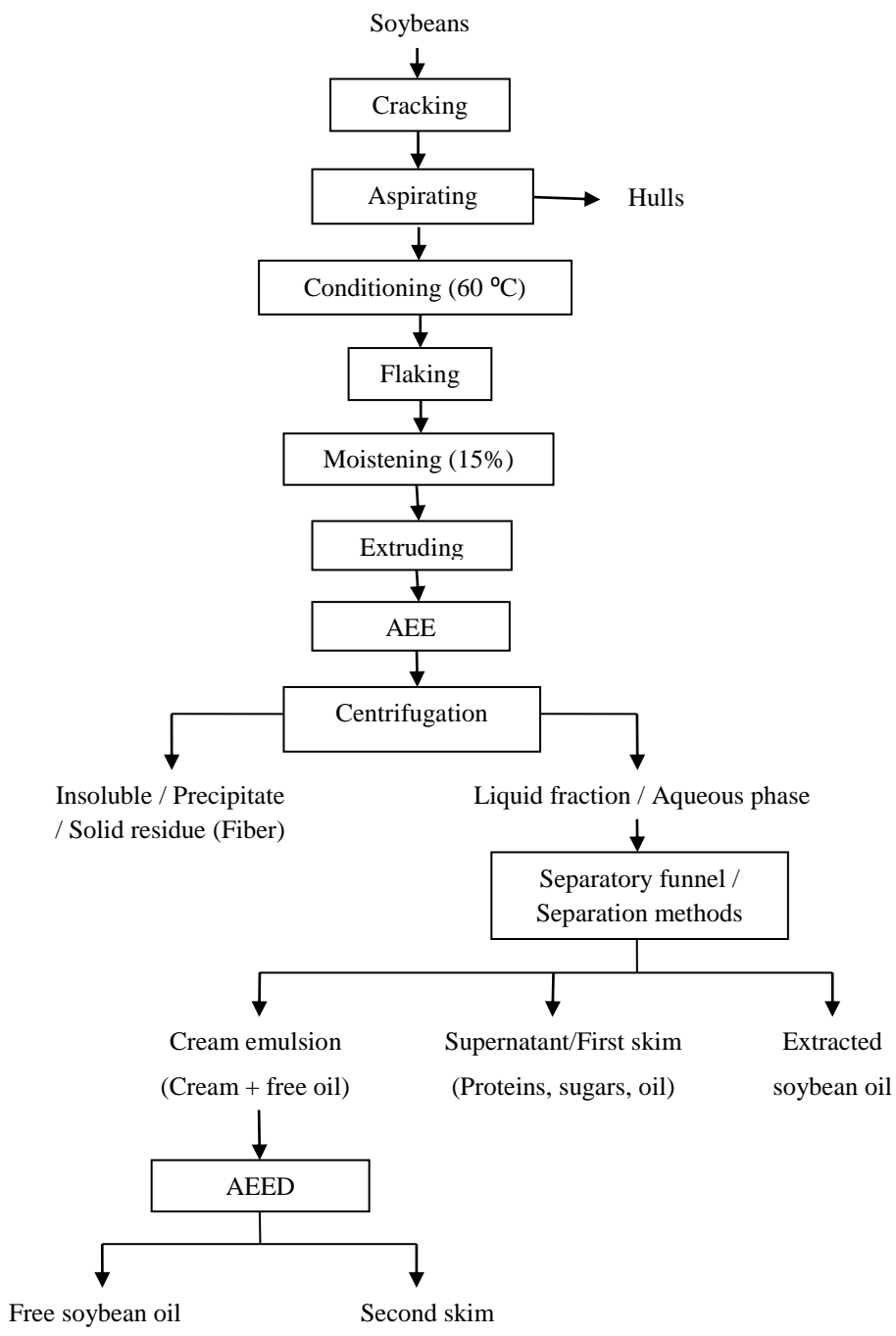
941 **Table 5. Maximum oil yields as affected by the selected and optimized incubating**
942 **conditions of the aqueous enzymatic extraction and aqueous enzymatic emulsion de-**
943 **emulsification methods.**

944 **Table 6. The characteristics of oil yields from solvent, aqueous, and aqueous**
945 **enzymatic extraction methods.**

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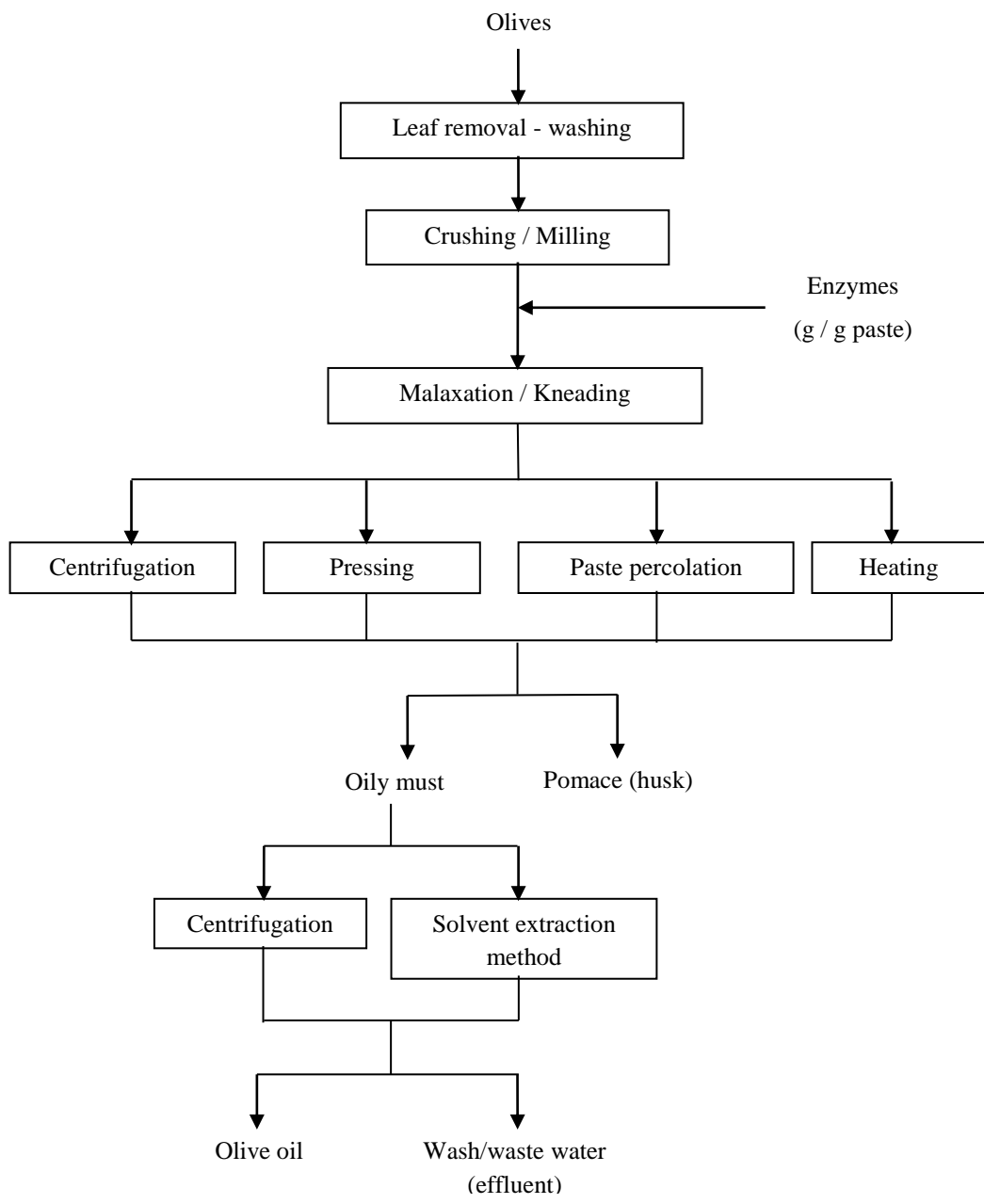
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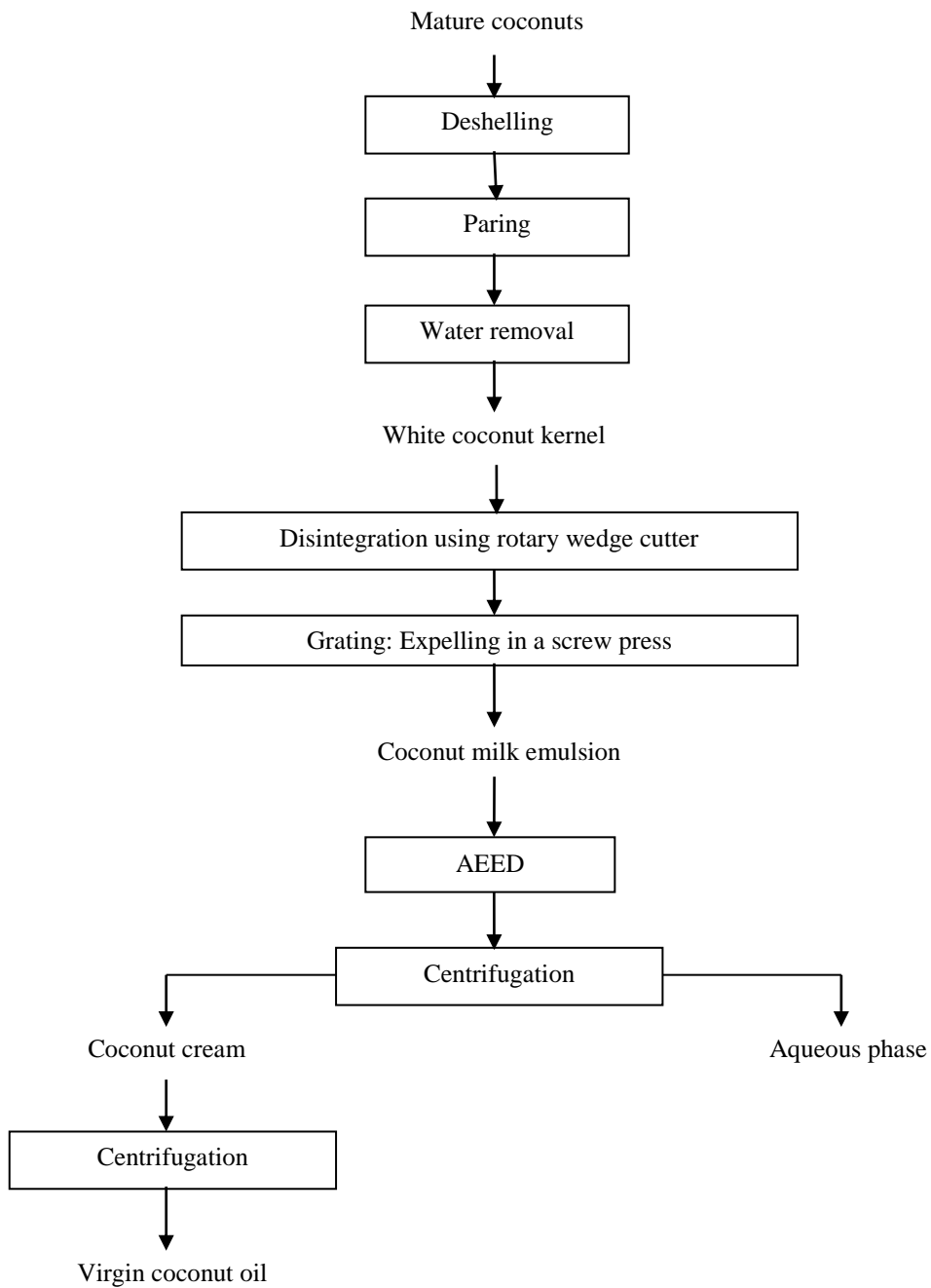
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Table 1. Commercial enzymes used for aqueous enzymatic extraction (AEE) and aqueous enzymatic emulsion de-emulsification (AEED) processes: descriptions and compositions.

| Enzymes commercial names | Description/Composition | Reference |
|---------------------------|----------------------------|--|
| <i>Single enzyme</i> | | |
| Alcalase® | Protease | Womeni <i>et al.</i> (2008) |
| Alcalase 2.4L | Protease | Rosenthal <i>et al.</i> (2001) Latif & Anwar (2009) Jiang <i>et al.</i> (2010) Latif & Anwar (2011) Rovaris <i>et al.</i> (2012) Tabtabaei & Diosady (2013) |
| As1398 | Protease | Jiang <i>et al.</i> (2010) |
| Celluclast 1.5L® | Cellulase | Dominguez <i>et al.</i> (1996) Sineiro <i>et al.</i> (1998) Abdulkarim <i>et al.</i> (2006) Rovaris <i>et al.</i> (2012) Tabtabaei & Diosady (2013) Teixeira <i>et al.</i> (2013) |
| Flavourzyme® 1000 L | Protease | Nyam <i>et al.</i> (2009) Nyam <i>et al.</i> (2009a) |
| Glucanex | Glucosidases | Garcia <i>et al.</i> (2001) |
| G-ZYME® G999 | Lysophospholipase A1 | Chabrand & Glatz (2009) Wu <i>et al.</i> (2009) Tabtabaei & Diosady (2013) |
| Lipomod 699L | Phospholipase A2 | Tabtabaei & Diosady (2013) |
| LysoMax™ | Phospholipase A2 | Wu <i>et al.</i> (2009) |
| Multifect Neutral® | Protease | Lamsal & Johnson (2007) |
| Neutrase 0.8L | Bacterial neutral protease | Abdulkarim <i>et al.</i> (2005) Abdulkarim <i>et al.</i> (2006) Nyam <i>et al.</i> (2009) Nyam <i>et al.</i> (2009a) |
| Nutrase | Xylanase | Jiang <i>et al.</i> (2010) |
| Papain | Protease | Jiang <i>et al.</i> (2010) |
| Pectinase 1.06021 | Pectinase | Najafian <i>et al.</i> (2009) |
| Pectinase Multieffect FE® | Pectinase | Teixeira <i>et al.</i> (2013) |

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|------------------------|--|-----------------------------------|
| Pectinex® | Pectinase | Womeni <i>et al.</i> (2008) |
| Pectinex Ultra SP | Pectinase | Dominguez <i>et al.</i> (1996) |
| Pectinex Ultra SP-L | Pectinase | Abdulkarim <i>et al.</i> (2006) |
| | | Tabtabaei & Diosady (2013) |
| Promozyme | Pullulanase | Shah <i>et al.</i> (2005) |
| Protamex | Protease | Jiang <i>et al.</i> (2010) |
| Protex 6L | Alkaline serine endopeptidase | Chabrand & Glatz (2009) |
| | | Jung <i>et al.</i> (2009) |
| | | Wu <i>et al.</i> (2009) |
| | | Shan Liu <i>et al.</i> (2011) |
| | | Xiaonan Sui <i>et al.</i> (2011) |
| | | Tabtabaei & Diosady (2013) |
| Protex 7L | Natural metallo endopeptidase | Latif <i>et al.</i> (2008) |
| | | Chabrand & Glatz (2009) |
| | | Jung & Mahfuz (2009) |
| | | Latif & Anwar (2009) |
| | | Wu <i>et al.</i> (2009) |
| | | Latif & Anwar (2011) |
| | | Latif <i>et al.</i> (2011) |
| Protex 30L | Alkaline serine endopeptidase | Chabrand & Glatz (2009) |
| Protex 50FP | Acid fungal endopeptidase- exopeptidase complex | Wu <i>et al.</i> (2009) |
| Protex 51FP | Neutral fungal endopeptidase- exopeptidase complex | Wu <i>et al.</i> (2009) |
| | | Tabtabaei & Diosady (2013) |
| Protex 89L | Endopeptidase | Tabtabaei & Diosady (2013) |
| ROHALASE® OS | Cellulase | Szydłowska-Czerniak <i>et al.</i> |
| ROHAPECT® PTE | Pectinase | (2010) |
| Termamyl 120L | α -amylase | Abdulkarim <i>et al.</i> (2006) |
| <i>Enzymes mixture</i> | | |
| Bioliva | Cellulase, hemicellulase, pectinase, other minor enzymes | Ranalli <i>et al.</i> (2003) |
| Cytolase 0 | Cellulase, hemicellulase, pectinase, other minor enzymes | Ranalli <i>et al.</i> (1999) |
| | | Ranalli <i>et al.</i> (2003) |
| Kemzyme | Cellulase complex, hemi-cellulase complex, α -amylase, β -glucanase, protease, xylanase | Latif & Anwar (2009) |
| | | Latif & Anwar (2011) |
| | | Latif <i>et al.</i> (2011) |

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|------------------------|---|---|
| Maxoliva | Cellulase, hemicellulase, pectinase, other minor enzymes | Ranalli <i>et al.</i> (2003) |
| Multifect CX 13L | Cellulase, hemicellulase, β -glucanase, arabinoxylans | Latif <i>et al.</i> (2008) Latif <i>et al.</i> (2011) |
| Multifect Pectinase FE | Pectinase, cellulase, hemicellulase | Latif <i>et al.</i> (2008) |
| Natuzyme | Cellulase, xylanase, phytase, α - amylase, pectinase | Latif <i>et al.</i> (2008) Latif & Anwar (2009) Latif & Anwar (2011) Latif <i>et al.</i> (2011) |
| Olivex | Cellulase, hemicellulase, pectinase | Garcia <i>et al.</i> (2001) |
| Olivex-Celluclast | 50%: Cellulase, hemicellulase pectinase 50%: Cellulase, hemicellulase | Soto <i>et al.</i> (2007) |
| Pectinex Ultra SP-L | Cellulase, pectinase, xylanase | Shah <i>et al.</i> (2005) Najafian <i>et al.</i> (2009) Tabatabaei & Diosady (2013) |
| Protizyme™ | Three different proteases with pH optima 3-4, 5-7, 7-10 | Sharma <i>et al.</i> (2002) Gaur <i>et al.</i> (2007) Jiang <i>et al.</i> (2010) |
| Rapidase® Liq plus | Hemicellulases, pectinases, cellulases | Gros <i>et al.</i> (2003) |
| Viscozyme® | (Carbohydrases): Cellulase, hemicellulase, arabinase, xylanase, amylase, β -glucanase | Sowbhagya <i>et al.</i> (2009) Womeni <i>et al.</i> (2008) |
| Viscozyme L | (Carbohydrases): Cellulase, hemicellulase, arabinase, xylanase, β - glucanase | Latif & Anwar (2009) Latif & Anwar (2011) Latif <i>et al.</i> (2011) Rovaris <i>et al.</i> (2012) Tabatabaei & Diosady (2013) |

Table 2. Oil yield difference between the aqueous and aqueous enzymatic extraction, and between solvent and aqueous enzymatic extraction methods.

| Oil-bearing material | Type of enzyme | Difference in oil yield (%) | | Reference |
|--|--|---|--|---------------------------------|
| | | Aqueous extraction and aqueous enzymatic extraction | Solvent treatment and aqueous enzymatic extraction | |
| Crushed borage seeds (≤ 2.0 mm) | Olivex / Celluclast (1:1) | 7.80 | - | Soto <i>et al.</i> (2007) |
| Extruded soybean flakes | Protease | 20.00 | - | Lamsal <i>et al.</i> (2006) |
| | Multifect Neutral® | 13.40 | - | Lamsal & Johnson (2007) |
| | Protex 7L | 22.10 | - | Jung & Mahfuz (2009) |
| | Protex 51FP | 16.00 ^a | - | Wu <i>et al.</i> (2009) |
| | Protex 6L | 20.00 ^a | - | |
| | Protex 7L | 17.00 ^a | - | |
| Ground canola seeds | Multifect CX 13L | 9.50 | 17.10 | Latif <i>et al.</i> (2008) |
| | Protex 7L | 6.90 | 19.70 | |
| | Natuzyme | 6.20 | 20.40 | |
| Ground <i>Jatropha</i> seed kernels (inedible) | Protizyme™ | 26.00 | | Shah <i>et al.</i> (2005) |
| Ground Kalahari melon seeds | Neutrased 0.8L | | 9.58 | Nyam <i>et al.</i> (2009a) |
| | Flavourzyme 1000L | | 8.67 | |
| Ground <i>Moringa. oleifera</i> seeds | Neutrased 0.8L | | 8.20 | Abdulkarim <i>et al.</i> (2005) |
| | Neutrased 0.8L | 12.12 | 9.39 | Abdulkarim <i>et al.</i> (2006) |
| | Termamyl 120L | 10.15 | 11.36 | |
| | Pectinex Ultra SP-L | 6.98 | 14.53 | |
| | Celluclast 1.5L | 10.12 | 11.39 | |
| | Neutrased 0.8L / Termamyl 120L / Pectinex Ultra SP-L / Celluclast 1.5L | 12.83 | 8.68 | |

| | | | | |
|------------------------------------|-------------------------|-------|-----------|--------------------------------|
| | Natuzyne | 9.10 | 23.30 | Latif <i>et al.</i> (2011) |
| | Kemzyme | 10.30 | 22.10 | |
| | Multifect CX 13L | 14.00 | 18.40 | |
| | Protex 7L | 14.70 | 17.70 | |
| | Viscozyme L | 13.10 | 19.30 | |
| Ground peanuts | Alcalase | 42.86 | - | Jiang <i>et al.</i> (2010) |
| | As1398 | 35.77 | - | |
| | Nutrase | 29.49 | - | |
| | Protizyme | 24.43 | - | |
| | Protamex | 18.30 | - | |
| | Protizyme TM | - | 3.36-5.88 | Sharma <i>et al.</i> (2002) |
| | Papain | - | 10.08 | |
| | Chymotrypsin | - | 16.38 | |
| | Trypsin | - | 13.86 | |
| Ground sesame seeds | Alcalase 2.4L | 12.50 | 25.40 | Latif & Anwar (2011) |
| | Natuzyne | 4.50 | 33.40 | |
| | Protex 7L | 6.40 | 31.50 | |
| | Viscozyme L | 9.10 | 28.80 | |
| | Kemzyme | 4.20 | 33.70 | |
| Ground sunflower seeds (0.75-1 mm) | Celluclast 1.5L | 35.00 | - | Sineiro <i>et al.</i> (1998) |
| Ground sunflower seeds | Alcalase 2.4L | 8.30 | 18.90 | Latif & Anwar (2009) |
| | Kemzyme | 13.90 | 13.30 | |
| | Natuzyne | 17.20 | 10.00 | |
| | Protex 7L | 10.00 | 17.20 | |
| | Viscozyme L | 21.40 | 5.80 | |
| Heat-treated soybean flour | Alcalase 2.4L | 16.90 | - | Rosenthal <i>et al.</i> (2001) |

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|---|--|-------------------|-------|----------------------------------|
| Kernel flour of bush mango | Alcalase® | 7.60 | - | Womeni <i>et al.</i> (2008) |
| | Pectinex® | 14.80 | - | |
| | Viscozyme® | 40.60 | - | |
| Minced yellow horn seed kernels | Cellulase / Hemicellulase / Pectinase (1.8 : 1.3 : 2.5) | | 9.00 | Li <i>et al.</i> (2013) |
| Olive paste | Bioliva | 1.20 | - | Ranalli <i>et al.</i> (2003) |
| | Maxoliva | 1.37 | - | |
| | Cytolase 0 | 1.44 | - | |
| | A (pectinase, cellulase, hemicellulase) | 152.00 (30 min) | - | Aliakbarian <i>et al.</i> (2008) |
| | / B (pectinase, hemicellulase) / | 91.40 (150 min) | - | |
| | C (pectolytic enzyme) (1:1:1) | | | |
| | Pectinex Ultra SP-L | 1.96 ^b | - | |
| | Pectinase 1.6021 | 1.41 ^b | - | |
| Palm fruit | Pectinase / cellulase | 35.57 | 5.36 | Teixeira <i>et al.</i> (2013) |
| | Pectinase / cellulase / tannase | 35.90 | 5.03 | |
| | Tannase | 12.70 | 28.23 | |
| | | | | |
| Rapeseed slurry | Pectinase | 38.10 | - | Zhang <i>et al.</i> (2007) |
| | Cellulase | 21.50 | - | |
| | B-glucanase | 16.20 | - | |
| | Pectinase / Cellulase / β -glucanase (4:1:1) | 43.80 | - | |
| | Multifect Pectinae FE | 5.70 | - | |
| Shattered bayberry kernels (60- mesh sieved) | Cellulase / Neutral protease (1:2) | | 31.85 | Zhang <i>et al.</i> (2012) |
| Yellow mustard flour | Celluclast 1.5L | 3.74 | 10.59 | Tabatabaei & Diosady (2013) |
| | Pectinex Ultra SP-L | 3.03 | 11.30 | |

| | | |
|---|------|-------|
| Viscozyme L | 3.99 | 10.34 |
| Celluclast 1.5L / Pectinex Ultra SP-L / Viscozyme L (1: 1:1) | 6.70 | 7.63 |

The oil yield differences were determined based on the oil yields under the best incubating conditions of each enzyme used, or based on the fixed incubating conditions for all enzymes used, in the conducted studies.

All aqueous enzymatic extractions resulted in higher oil yields than aqueous extractions, and all solvent treatments resulted in higher oil yields than aqueous enzymatic extractions.

^a total oil as in the skim and cream emulsion

^b average oil yield enhancements from three olive species with the use of enzymes at high concentrations

Table 3. Enhancement in oil yield due to presence of enzyme pre-treatment prior to the extraction method, as compared to the extraction method alone.

| Oil-bearing material | Type of enzyme (pre-treatment) | Extraction method | Enhancement in oil yield (%) | Reference |
|--|---|--|---|--|
| Crushed borage seeds (≤ 2.0 mm) | Olivex / Celluclast (1:1) | Double pressing | 5.40 ^a | Soto <i>et al.</i> (2007) |
| Crushed garlic cloves | Cellulase | Steam distillation | 0.11 | Sowbhagya <i>et al.</i> (2009) |
| | Pectinase | | 0.23 | |
| | Protease | | 0.22 | |
| | Viscozyme | | 0.18 | |
| | Cellulase | Hydrodistillation | 0.14 | |
| | Pectinase | | 0.26 | |
| | Protease | | 0.24 | |
| | Viscozyme | | 0.19 | |
| Ground flaxseeds | Cellulase / Pectinase / Hemicellulase (1:1:1) | Ultrasonication | 29.50 | Long <i>et al.</i> (2011) |
| Ground rapeseeds | ROHAPECT® PTE | Pressing | 5.70 | Szydłowska-Czerniak <i>et al.</i> (2010) |
| | ROHALASE® OS | | 1.70 | |
| Milled grape seeds | A mixture of cellulase, xylanase, protease, pectinase | Solvent extraction (24 hr) | 106.00 | Passos <i>et al.</i> (2009) |
| | | Solvent extraction (120 hr) | 163.00 | |
| Minced yellow horn seed kernels | Cellulase / hemicellulase / pectinase (1.8 : 1.3 : 2.5) | Microwave | 4.30 (oil yield enhancement as compared to AEE alone) | Li <i>et al.</i> (2013) |
| Pre-heated ground Chilean hazelnut seeds (inedible, ≤ 1.4 mm) | Ultrazyme / Celluclast (1:1) | Double pressing (hydraulic pressing at each of 39.2 MPa) | ~8.00 | Zuniga <i>et al.</i> (2003) |
| <i>Silybum marianum</i> seed powders | Cellulase / Xylanase / Pectinase / Protease (2:1:1:2) | Solvent extraction (1.5 hr) | 10.46 | Li <i>et al.</i> (2012) |
| | | Solvent extraction (14.0 hr) | 50.72 | |
| Whole sunflower kernels | Celluclast 1.5L / Pectinex Ultra SP (2:1) | Pressing (Batch press) | 13.11 | Dominguez <i>et al.</i> (1996) |
| Mango kernel powders | Protizyme™ | Three-phase partitioning method | 16.00 | Gaur <i>et al.</i> (2007) |
| Soybean flour | | | 8.00 | |

Rice bran powders

14.00

^a the oil yield enhancement was based on the difference between an enzymatic and non-enzymatic pre-treatment, followed by double pressing

Table 4. The advantages of the use of pre-treatments (non-enzymatic) prior to the enzymatic extraction method.

| Oil-bearing material | Pre-treatment | Type of enzyme | Advantages | Reference |
|--|---|--|---|---|
| Ground <i>Isatis indigotica</i> seeds | Microwave | Cellulase / Proteinase / Pectinase (1:1:1) | - In combination with AEE, the use of optimal microwave irradiation power increased the oil yield up to 59.27%, and the oil yield had greater antioxidant properties than solvent-extracted oil. | Gai <i>et al.</i> (2013) |
| Ground <i>Jatropha</i> seed kernels (inedible) | Ultrasonication (5 min) | Protizyme™ | The enzyme treatment time was reduced from 18 hr to 6 hr for maximum of 74% oil yield | Shah <i>et al.</i> (2005) |
| Ground linseeds | Electrical discharge | - | Mucilage (stabilizing agent) is removed which caused easier oil separation from the resulted residue by using enzyme treatment | Gros <i>et al.</i> (2003) |
| Grounds peanuts | Alkaline extraction | Alcalase | Oil yield of 5.87% higher than AEE alone | Jiang <i>et al.</i> (2010) |
| Ground pitaya seeds (40-mesh sieved) | Microwave | Pectinase / Cellulase / Acid protease (1:1:1) | - Oil yield of 0.84% higher than AEE alone | Rui <i>et al.</i> (2009) |
| Ground watermelon kernels | Ultrasound | Protex 6L | -Under the fixed parameters of the ultrasound, the yield was 20.67% higher than AEE alone -Under the selected parameters of ultrasound for maximum oil yield, the yield was 21.39% higher than AEE alone | Xiaonan Sui <i>et al.</i> (2011), Shan Liu <i>et al.</i> (2011) |
| Soybean flakes | High pressure processing (200 MPa) High pressure processing (500 MPa) Extrusion | Protex 7L | Oil yield of 3.20% higher than AEE alone Oil yield of 1.30% higher than AEE alone - Oil yield of 29.90% higher than AEE alone - Free oil yield of 17.00% higher than AEE | Jung & Mahfuz (2009) |

| | | | |
|-----------|-----------|--|---------------------------|
| Extrusion | Protex 6L | alone - Oil yield of 35.52% higher than AEE alone - After de-emulsification: Free oil from cream emulsion of 62.00% higher than AEE alone | Jung <i>et al.</i> (2009) |
|-----------|-----------|--|---------------------------|

AEE: aqueous enzymatic extraction.

Table 5. Maximum oil yields as affected by the selected and optimized incubating conditions of the aqueous enzymatic extraction and aqueous enzymatic emulsion de-emulsification methods.

| Oil-bearing material | Type of enzyme | Moisture / Material ratio (w/w; for aqueous enzymatic extraction) | Enzyme / Material ratio | pH | Tempera- ture (°C) | Time (hr) | Agitation rate (rpm) | Oil yield (%) | Reference |
|--|--|--|-------------------------------|---------|-----------------------|-----------|-------------------------|------------------|---------------------------------|
| <i>Selected(*) and optimized (**) incubating conditions used for maximum oil yield in aqueous enzymatic extraction</i> | | | | | | | | | |
| Crushed borage seeds (≤2.0 mm) | Olivex / Celluclast (1:1) ^a | 20%* (corresponded to 1:5) | 0.25%* | - | 45.0* | 9.00* | - | 85.50 | Soto <i>et al.</i> (2007) |
| Ground <i>Jatropha</i> seed kernels (inedible) | Protizyme ^{TM a} | 6:1 | 0.25 (w/w)% | 9.00* | 50.0* | 18.00 | 100 | 64.00 | Shah <i>et al.</i> (2005) |
| Ground <i>Moringa.</i> <i>oleifera</i> seeds | Celluclast 1.5L ^a | 6:1 | 2.00% | 4.80*** | 60.0* | 36.00* | 120* | 22.01 | Abdulkarim <i>et al.</i> (2006) |
| | Termamyl 120L ^a | | (v/w)* | 5.50*** | | | | 22.04 | |
| | Pectinex Ultra SP-L ^a | | | 3.50*** | | | | 18.87 | |
| | Neutrased 0.8L ^a | | | 6.80*** | | | | 24.02 | |
| | Neutrased 0.8L / Termamyl 120L / Pectinex Ultra SP-L / Celluclast 1.5L ^a | | | 7.50*** | | | | 24.72 | |
| Ground peanuts | Alcalase ^a | 5:1* | 1.50% (w/w)* | 8.50* | 60.0* | 5.00* | - | 73.45 | Jiang <i>et al.</i> (2010) |

| | | | | | | | | | |
|---|---|-----------|------------------|----------|---------|-----------------|------------------|-----------------|-------------------------------------|
| | Protizyme TM ^a | 2:1 | 2.50% (w/w)* | 4.00* | 40.0* | 18.00* | 80* | 36.12- 38.64 | Sharma <i>et al.</i> (2002) |
| Ground pitaya seeds (40-mesh sieved) | Pectinase / Cellulase / Acid protease (1:1:1) ^a | 8:1 | - | 7.00 | 50.0* | 1.00 | 90 | 6.94 | Rui <i>et al.</i> (2009) |
| Ground rice bran (16-mesh sieved) | Alcalase 0.6L ^a | - | 1.00% (w/w)* | 9.00 | 60.0* | 3.00* | 1000 | 79.10 | Hanmoungjai <i>et al.</i> (2001) |
| Ground sunflower seeds (0.75-1 mm) | Celluclast 1.5L ^a | 5:1* | 2.00% (w/w)* | 4.80*** | 50.0*** | 2.00* | 150 | 35.65 | Sineiro <i>et al.</i> (1998) |
| Heat-treated soybean flour | Alcalase 2.4L ^a | - | 3.00% (v/w)* | 8.00 *** | 50.0*** | 1.00 | 200 | 58.70 | Rosenthal <i>et al.</i> (2001) |
| Olive paste | A (pectinase, cellulase, hemicellulase) / B (pectinase, hemicellulase) / C (pectolytic enzyme) (1:1:1) ^a | - | 0.25% (v/w)* | - | 30.0 | 2 hr 30 min* | 10 (kneading) | 17.50 | Aliakbarian <i>et al.</i> (2008) |
| Rapeseed slurry | Pectinase / Cellulase / β -glucanase (4:1:1) ^a | 5:1* | 2.50% (v/w)* | 5.00 | 48.0 | 4.00* | 200 | 92.70 | Zhang <i>et al.</i> (2007) |
| Ground Kalahari melon seeds | Neutrase 0.8L ^a | - | 2.50% (w/w)** | 7.00** | 58.0** | 31.00** | 100 | 68.58 | Nyam <i>et al.</i> (2009a) |
| | Flavourzyme® 1000 L ^a | - | 2.10% (w/w)** | 6.00** | 50.0** | 36.00** | 100 | 71.55 | |
| Ground <i>Moringa oleifera</i> seeds | Neutrase 0.8L ^a | 6:1 (v/w) | 2.00% (v/w) | 6.80 *** | 45.0** | 24.00** | 120 | 22.60 | Abdulkarim <i>et al.</i> (2005) |

| | | | | | | | | | |
|--|---|---|------------------|--------|---------|---------|-----|-------|--|
| Ground pine kernels | Alcalase endo- protease ^a | 5:1** | 1.97%** | 8.40** | 51.0** | 3.00** | - | 89.12 | Yang Li <i>et al.</i> (2011) |
| Ground pumpkin seeds | Cellulase ^a | - | 1.70% (w/w)** | - | 47.0** | 2.64** | - | 89.12 | Hu & Zou (2013) |
| Ground watermelon kernels | Protex 6L ^a | 4.35:1** | 2.63%** | 7.89** | 47.1** | 4.29** | - | 77.25 | Xiaonan Sui <i>et al.</i> (2011); Shan Liu <i>et al.</i> (2011) |
| Palm fruits | Pectinase / Cellulase / Tannase (1:1:1) ^a | 2:1 (v/w)** | 4.00** | 4.00** | 50.0 | 0.50* | 200 | 91.52 | Teixeira <i>et al.</i> (2013) |
| Shattered bayberry kernels (60-mesh sieved) | Cellulase / Neutral protease (1:2) ^a | 4.91:1 (v/w)** | 3.17%** | - | 51.6** | 4.00** | - | 31.15 | Zhang <i>et al.</i> (2012) |
| <i>Selected (*) and optimized (**) incubating conditions for maximum free oil yield in aqueous enzymatic emulsion de-emulsification method</i> | | | | | | | | | |
| Alkaline pre-treated ground peanuts | Alcalase 2.4L ^a | As1398 ^b | 1.00% | - | - | 2.0 hr | - | 12.66 | Jiang <i>et al.</i> (2010) |
| Coconut milk emulsion | - | Aspartic protease (endoprotease) ^b | 0.10% | - | 37.0* | 3.0 hr | - | 83.00 | Raghavendra & Raghavarao (2010) |
| Extruded soybean flakes | Protease Multifect Neutral ^a | LysoMax TM / | - | 4.5*** | 60.0*** | 1 hr 30 | - | 68.00 | Lamsal & Johnson (2007) |
| | | G-ZYME G999 (1:1) ^b Phospholipase | - | 7.0*** | 37.0*** | 1 hr 30 | - | 73.00 | |

| | | C ^b | | | | | | min | | |
|--|--|----------------------------|---------|------------|----------|---------------|------|--------|-------------------------------|--|
| | Protex 6L ^a | Protex 6L ^b | 2.50%* | 4.5* | 50.0 | 1 hr 30 min | - | 100.00 | de Moura <i>et al.</i> (2008) | |
| | Protex 6L ^a | Protex 6L ^b | 1.25%** | - | 50.0** | 1 hr 30 min** | - | 100.00 | Jung <i>et al.</i> (2009) | |
| | Protex 7L ^a | LysoMax™ ^b | 2.00% | 8.0*** | 40.0*** | 1 hr 30 min | - | 100.00 | Wu <i>et al.</i> (2009) | |
| | | G-ZYME® G999 ^b | | 4.5*** | 50.0*** | | | | | |
| | | Protex 6L ^b | | 8.0*** | 50.0*** | | | | | |
| | | Protex 7L ^b | | 7.0*** | 50.0*** | | | | | |
| | | Protex 50FP ^b | | 4.5*** | 50.0*** | | | | | |
| | | Protex 51FP ^b | | 8.0*** | 50.0*** | | | | | |
| Ground <i>Perilla frutescens</i> seeds | - | Protex 6L ^b | 1.90%** | 9.4** | 62.6** | 1.6 hr** | - | 85.52 | Zhang <i>et al.</i> (2013) | |
| Soybean flour | Protex 7L ^a | G-ZYME G999 ^b | 2.00%* | 4.5*** | 50.0 | 3.0 hr | 700* | 100.00 | Chabrand & Glatz (2009) | |
| | | Protex 6L ^b | 3.00%* | 9.0*** | 50.0 | 3.0 hr | 500* | 72.00 | | |
| Yellow mustard flour | Celluclast 1.5L / Viscozyme L / Pectinex Ultra SP-L (1:1:1) ^a | Protex 6L ^b | 2.50% | 4.5-6.0*** | 50-60*** | 3.0 hr | - | 91.30 | Tabtabaei & Diosady (2013) | |
| | | Alcalase 2.4L ^b | | 6.5-8.5*** | 45-65*** | | | 42.10 | | |
| | | Lipomode 699L ^b | | 8.0*** | 40.0*** | | | 1.30 | | |

| | | | |
|-------------------|--------|----------|-------|
| G-ZYME | 4.5*** | 50-60*** | 41.20 |
| G999 ^b | | | |

Values without any notation are fixed incubating conditions.

^a Type of enzymes used for aqueous enzymatic extraction

^b Type of enzymes used for aqueous enzymatic emulsion de-emulsification

*selected incubating condition; the authors varied the level of each incubating condition and finalized the conditions which resulted in highest oil yield.

**optimized incubating condition; the authors varied the level of each incubating condition and optimized the conditions which resulted in highest oil yield based on an experimental design and statistical software used.

*** optimum incubating condition of the enzyme used; different types of enzymes possess different optimum pH and temperature where the enzymes attain maximum activity

Table 6. The characteristics of oil yields from solvent, aqueous, and aqueous enzymatic extraction methods.

| Oil characteristic | Oil-bearing material | Solvent extraction | Aqueous extraction | Aqueous enzymatic extraction | Reference | | | | | |
|------------------------|-----------------------------------|--|--------------------|---------------------------------------|-----------------------|----------------------------------|---|----------------------------|---------------------|---------------------------------|
| Free fatty acids (%) | Extruded soybean flakes | 0.26 | * | 0.18 | Protex 6L | Jung <i>et al.</i> (2009) | | | | |
| | | | | 0.52 | Multifect CX 13L | | | | | |
| | Ground canola seeds | 0.81 | 0.56 | 0.57 | Protex 7L | Latif <i>et al.</i> (2008) | | | | |
| | | | | 0.55 | Natuzyme | | | | | |
| | | | | 0.54 | Multifect Pectinae FE | | | | | |
| | | | | 0.90 | Flavourzyme® 1000 L | | | | | |
| | | | | 0.90 | Neutrased 0.8L | | | | | |
| | | | | Ground Kalahari melon seeds | 0.60 | | * | 1.13 | Neutrased 0.8L | Nyam <i>et al.</i> (2009) |
| | | | | | | | | 1.13 | Neutrased 0.8L | |
| | | | | Ground <i>Moringa. oleifera</i> seeds | 2.48 | | * | 1.22 | Neutrased 0.8L | Abdulkarim <i>et al.</i> (2005) |
| | | | | | | | | 1.24 | Termamyl 120L | |
| | | | | | | | | 1.22 | Pectinex Ultra SP-L | |
| | 1.25 | Celluclast 1.5L | | | | | | | | |
| | 1.23 | Neutrased 0.8L / Termamyl 120L / Pectinex Ultra SP-L / Celluclast 1.5L | | | | | | | | |
| | 1.26 | 0.42 | 0.43 | | | Natuzyme | | Latif <i>et al.</i> (2011) | | |
| | 0.41 | Kemzyme | | | | | | | | |
| | 0.39 | Multifect CX 13L | | | | | | | | |
| | Ground rice bran (16-mesh sieved) | 7.40 | * | 0.38 | Protex 7L | Hanmoungjai <i>et al.</i> (2001) | | | | |
| | | | | 0.42 | Viscozyme L | | | | | |
| | Ground sesame seeds | 0.54c | 0.48 | 2.36 | Alcalase 0.6L | Latif & Anwar (2011) | | | | |
| 0.47 | | | | Natuzyme | | | | | | |
| 0.44 | | | | Kemzyme | | | | | | |
| 0.51 | | | | Protex 7L | | | | | | |
| 0.46 | | | | Alcalase 2.4L | | | | | | |
| Ground sunflower seeds | 0.94 | 0.68 | 0.44 | Viscozyme L | Latif & Anwar (2009) | | | | | |
| | | | 0.66 | Alcalase 2.4L | | | | | | |
| | | | 0.65 | Kemzyme | | | | | | |

| | | | | | | | | |
|----------------------------|--|--------|--------|---------------|--|-------------------------------------|----------|-------------------------------|
| | | | | 0.67 | Natuzyme | | | |
| | | | | 0.69 | Protex 7L | | | |
| | | | | 0.64 | Viscozyme L | | | |
| Iodine value (g / 100g) | Ground canola seeds | 117.00 | 114.00 | 116.00 | Multifect CX 13L | Latif <i>et al.</i> | | |
| | | | | 114.00 | Protex 7L | (2008) | | |
| | | | | 117.00 | Natuzyme | | | |
| | | | | 116.00 | Multifect Pectinae FE | | | |
| | Ground flaxseeds | 140.80 | * | 161.20 | Cellulase / Pectinase / Hemicellulase (1:1:1) | Long <i>et al.</i> (2011) | | |
| | Ground Kalahari melon seeds | 125.00 | * | 141.00 | Flavourzyme® 1000 L | Nyam <i>et al.</i> (2009) | | |
| | Ground <i>Moringa</i> . <i>oleifera</i> seeds | 65.40 | * | 66.10 | Neutrase 0.8L | Abdulkarim <i>et al.</i> (2005) | | |
| | | | | 67.10 | Neutrase 0.8L | Abdulkarim <i>et al.</i> (2006) | | |
| | | | | 66.50 | Termamyl 120L | | | |
| | | | | 67.20 | Pectinex Ultra SP-L | | | |
| | | | | 66.50 | Celluclast 1.5L | | | |
| | | | | 67.00 | Neutrase 0.8L / Termamyl 120L / Pectinex Ultra SP-L / Celluclast 1.5L | | | |
| | | | | 67.00 | 70.00 | 76.00 | Natuzyme | Latif <i>et al.</i> (2011) |
| | | | | | | | 73.00 | Kemzyme |
| | | | | 75.00 | Multifect CX 13L | | | |
| | | | | 74.00 | Protex 7L | | | |
| | | | | 76.00 | Viscozyme L | | | |
| | Ground pitaya seeds (40-mesh sieved) | 173.10 | * | 118.00 | Pectinase / Cellulase / Acid protease (1:1:1) | Rui <i>et al.</i> (2009) | | |
| | Ground rice bran (16- mesh sieved) | 95.40 | * | 97.18 | Alcalase 0.6L | Hanmoungjai <i>et al.</i> (2001) | | |
| | Ground sesame seeds | 107.00 | 106.00 | 104.00 | Natuzyme | Latif & Anwar (2011) | | |
| 109.00 | | | | Kemzyme | | | | |
| 108.00 | | | | Protex 7L | | | | |
| 105.00 | | | | Alcalase 2.4L | | | | |
| 103.00 | | | | Viscozyme L | | | | |
| Ground sunflower | 127.00 | 120.00 | 124.00 | Alcalase 2.4L | Latif & | | | |

| | | | | | | |
|--|---------------------------------------|--------|------|--------|---|----------------------------------|
| | seeds | | | 121.00 | Kemzyme | Anwar (2009) |
| | | | | 123.00 | Natuzyne | |
| | | | | 122.00 | Protex 7L | |
| | | | | 121.00 | Viscozyme L | |
| Peroxide value (meq O ₂ / kg) | Extruded soybean flakes | 6.50 | * | 4.05 | Protex 6L | Jung <i>et al.</i> (2009) |
| | Ground canola seeds | 1.29 | 0.69 | 0.72 | Multifect CX 13L | Latif <i>et al.</i> (2008) |
| | | | | 0.70 | Protex 7L | |
| | | | | 0.71 | Natuzyne | |
| | | | | 0.64 | Multifect Pectinae FE | |
| | Ground flaxseeds | 1.20 | * | 1.00 | Cellulase / Pectinase / Hemicellulase (1:1:1) | Long <i>et al.</i> (2011) |
| | Ground Kalahari melon seeds | 2.30 | * | 6.40 | Flavourzyme® 1000 L | Nyam <i>et al.</i> (2009) |
| | Ground <i>Moringa. oleifera</i> seeds | 2.09 | 1.60 | 7.30 | Neutrased 0.8L | |
| | | | | 1.58 | Natuzyne | Latif <i>et al.</i> (2011) |
| | | | | 1.56 | Kemzyme | |
| | | | | 1.61 | Multifect CX 13L | |
| | | | | 1.63 | Protex 7L | |
| | | | | 1.59 | Viscozyme L | |
| | Ground pitaya seeds (40-mesh sieved) | 1.93 | * | 1.44 | Pectinase / Cellulase / Acid protease (1:1:1) | Rui <i>et al.</i> (2005) |
| | Ground rice bran (16-mesh sieved) | 8.20 | * | 12.01 | Alcalase 0.6L | Hanmoungjai <i>et al.</i> (2001) |
| | Ground sesame seeds | 1.50 | 1.30 | 0.90 | Natuzyne | Latif & Anwar (2011) |
| | | | | 1.30 | Kemzyme | |
| | | | | 1.40 | Protex 7L | |
| | | | | 1.10 | Alcalase 2.4L | |
| | | | | 1.20 | Viscozyme L | |
| | Ground sunflower seeds | 1.78 | 1.36 | 1.25 | Alcalase 2.4L | Latif & Anwar (2009) |
| | | | | 1.33 | Kemzyme | |
| | | | | 1.32 | Natuzyne | |
| | | | | 1.31 | Protex 7L | |
| | | | | 1.37 | Viscozyme L | |
| Saponification value | Ground Kalahari melon seeds | 173.20 | * | 185.20 | Flavourzyme® 1000 L | Nyam <i>et al.</i> (2009) |
| | | | | 184.80 | Neutrased 0.8L | |

| | | | | | | |
|--|---------------------------------------|-----------------------------------|--------|---------------|---|---------------------------------|
| (mg KOH / g oil) | Ground <i>Moringa. oleifera</i> seeds | 164.00 | * | 163.00 | Neutrased 0.8L | Abdulkarim <i>et al.</i> (2005) |
| | | 164.00 | 158.00 | 156.00 | Natuzyne | Latif <i>et al.</i> (2011) |
| | | | | 158.00 | Kemzyme | |
| | | | | 155.00 | Multifect CX 13L | |
| | | | | 159.00 | Protex 7L | |
| | 156.00 | Viscozyme L | | | | |
| | Ground pitaya seeds (40-mesh sieved) | 194.40 | * | 191.10 | Pectinase / Cellulase / Acid protease (1:1:1) | Rui <i>et al.</i> (2005) |
| | | Ground rice bran (16-mesh sieved) | 187.60 | * | 188.72 | Alcalase 0.6L |
| | 158.00 | | | | Natuzyne | Latif & Anwar (2011) |
| | 162.00 | | | | Kemzyme | |
| | 167.00 | | | | Protex 7L | |
| | Ground sesame seeds | 169.00 | 159.00 | 158.00 | Natuzyne | |
| | | | | 162.00 | Kemzyme | |
| | | | | 167.00 | Protex 7L | |
| | | | | 164.00 | Alcalase 2.4L | |
| 156.00 | | | | Viscozyme L | | |
| Ground sunflower seeds | 190.00 | 187.00 | 187.00 | Alcalase 2.4L | Latif & Anwar (2009) | |
| | | | 186.00 | Kemzyme | | |
| | | | 187.00 | Natuzyne | | |
| | | | 187.00 | Protex 7L | | |
| | | | 185.00 | Viscozyme L | | |
| Total tocopherols; α , δ , and γ (α , β , δ , and γ for Kalahari melon seeds and olive paste) (mg / kg oil) | Ground canola seeds | 739.00 | 598.00 | 794.00 | Multifect CX 13L | Latif <i>et al.</i> (2008) |
| | | | | 805.00 | Protex 7L | |
| | | | | 783.00 | Natuzyne | |
| | Ground Kalahari melon seeds | 174.80 | * | 143.20 | Flavourzyme® 1000 L | Nyam <i>et al.</i> (2009) |
| | | | | 143.30 | Neutrased 0.8L | |
| | Ground <i>Moringa oleifera</i> seeds | 179.30 | 216.90 | 220.80 | Natuzyne | Latif <i>et al.</i> (2011) |
| | | | | 228.50 | Kemzyme | |
| | | | | 221.70 | Multifect CX 13L | |
| | | | | 221.50 | Protex 7L | |
| | | | | 228.30 | Viscozyme L | |
| Ground sesame seeds | 584.10 | 603.30 | 628.50 | Natuzyne | Latif & Anwar (2011) | |
| | | | 641.20 | Kemzyme | | |
| | | | 627.30 | Protex 7L | | |
| | | | 619.80 | Alcalase 2.4L | | |

| | | | | | | |
|--|--------------------------------------|--------------|--------|--------|---------------------------------|-------------------------------|
| | | | | 612.80 | Viscozyme L | |
| | Ground sunflower seeds | 799.00 | 778.00 | 845.00 | Alcalase 2.4L | Latif & Anwar (2009) |
| | | | | 849.00 | Kemzyme | |
| | | | | 849.00 | Natuzyme | |
| | | | | 842.00 | Protex 7L | |
| | | | | 833.00 | Viscozyme L | |
| | Olive paste | Cipressino * | 77.30 | 89.20 | Cytolase 0 | Ranalli <i>et al.</i> (2001) |
| | | Cassanese | 95.20 | 114.10 | | |
| | | Leccino | 117.00 | 135.40 | | |
| | | Dritta * | 231.00 | 288.00 | Cytolase 0 | Ranalli <i>et al.</i> (2003) |
| | | | | 279.00 | Maxoliva | |
| | | | | 266.00 | Bioliva | |
| | | Caroleo * | 218.00 | 273.00 | Cytolase 0 | |
| | | | | 269.00 | Maxoliva | |
| | | | | 252.00 | Bioliva | |
| | | Coratina * | 244.00 | 305.00 | Cytolase 0 | |
| | | | | 300.00 | Maxoliva | |
| | | | | 289.00 | Bioliva | |
| | Palm fruit | * | 325.27 | 251.11 | Pectinase / Cellulase | Teixeira <i>et al.</i> (2013) |
| | | | | 200.54 | Pectinase / Cellulase / Tannase | |
| | | | | 204.26 | Tannase | |
| Total phenolic content (mg / kg oil), as in gallic acid equivalent for sesame seeds, sunflower seeds, <i>Moringa oleifera</i> seeds, and palm fruit; | Ground Kalahari melon seeds | 18.00 | * | 18.00 | Flavourzyme® 1000 L | Nyam <i>et al.</i> (2009) |
| | Ground <i>Moringa oleifera</i> seeds | 12.00 | 13.00 | 19.00 | Neutrase 0.8L | Latif <i>et al.</i> (2011) |
| | | | | 15.00 | Natuzyme | |
| | | | | 14.00 | Kemzyme | |
| | | | | 13.00 | Multifect CX 13L | |
| | | | | 14.00 | Protex 7L | |
| | | | | 18.00 | Viscozyme L | |
| | Ground sesame seeds | 17.00 | 18.00 | 19.00 | Natuzyme | Latif & Anwar (2011) |
| | | | | 18.00 | Kemzyme | |
| | | | | 22.00 | Protex 7L | |
| | | | | 21.00 | Alcalase 2.4L | |
| | | | | 24.00 | Viscozyme L | |
| | Ground sunflower seeds | 8.00 | 9.00 | 13.00 | Alcalase 2.4L | Latif & Anwar (2009) |
| | | | | 14.00 | Kemzyme | |

| | | | | | | | |
|----------------|------------|------------|---|--------|--------|-------------------------|-------------------------------------|
| caffeic acid | | | | 13.00 | | Natuzyme | |
| equivalent for | | | | 13.00 | | Protex 7L | |
| olive paste; | | | | 15.00 | | Viscozyme L | |
| and sum of | Olive | Cipressino | * | 90.00 | 105.00 | Cytolase 0 | Ranalli <i>et al.</i> |
| phenolic | paste | Cassanese | | 122.00 | 153.00 | | (2001) |
| acids for | | Leccino | | 112.00 | 131.00 | | |
| Kalahari | | Dritta | * | 314.00 | 435.00 | Cytolase 0 | Ranalli <i>et al.</i> |
| melon seeds | | | | | 427.00 | Maxoliva | (2003) |
| | | | | | 388.00 | Bioliva | |
| | | Caroleo | * | 222.00 | 329.00 | Cytolase 0 | |
| | | | | | 318.00 | Maxoliva | |
| | | | | | 287.00 | Bioliva | |
| | | Coratina | * | 382.00 | 479.00 | Cytolase 0 | |
| | | | | | 462.00 | Maxoliva | |
| | | | | | 431.00 | Bioliva | |
| | | Coratina | * | 691.30 | 751.00 | A / B / C** (1:1:1) | Aliakbarian <i>et al.</i> (2008) |
| | | Coratina | * | 574.50 | 804.30 | A / B / C** (1:1:1) | De Faveri <i>et al.</i> (2008) |
| | | Koroneiki | * | 179.00 | 309.00 | Pectinex | Najafian <i>et al.</i> (2009) |
| | | | | | 245.00 | Pectinase | |
| | | Iranian | * | 302.33 | 357.67 | Pectinex | |
| | | oleaginous | | | 359.00 | Pectinase | |
| | | Mission | * | 199.67 | 306.67 | Pectinex | |
| | | | | | 258.33 | Pectinase | |
| | Palm fruit | | * | 21.43 | 17.43 | Pectinase / Cellulase | Teixeira <i>et al.</i> |
| | | | | | 14.76 | Pectinase / Cellulase / | (2013) |
| | | | | | | Tannase | |
| | | | | | 26.43 | Tannase | |

The column adjacent to the olive paste refers to the different olive species used.

*data not reported

**A: pectinase, cellulase, hemicellulase; B: pectinase, hemicellulase; C: pectolytic enzyme